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Archives of Internal Medicine

VOLUME 39 FEBRUARY, 1927 NUMBER 2

DETERMINATION OF BRONCHOSPASM IN THE GUINEA-PIG

APPLICATIONS OF THE METHOD *

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AND
JULIAN H. LEWIS, M.D., PH.D.
CHICAGO

In continuation of studies on the production of amines [1] by bacteria, we desired physiologic methods to supplement the chemical methods that are now being used in this laboratory [2] for the assay of bacterial filtrates. The marked action of amines on smooth muscle, causing violent contractions, forms the basis of such physiologic methods. The adaptation of this action on the smooth muscle of isolated arteries provided one such method, the technic of which is described in a later article. The present article is concerned with still another method, based on the action of amines on the smooth muscle of the bronchi.

* From the Otho S. A. Sprague Memorial Institute for Medical Research and the department of pathology of the University of Chicago.

1. Koessler, K. K., and Hanke, M. T.: Studies on Proteinogenous Amines: IV, The Production of Histamine from Histidine by Bacillus Coli Communis, J. Biol. Chem. **39**:539-584 (Oct.) 1919; Studies on Proteinogenous Amines: XII, The Production of Histamine and Other Imidazoles from Histidine by the Action of Micro-Organisms, ibid. **50**:133-191 (Jan.) 1922; Studies on Proteinogenous Amines: XVII, On the Faculty of Normal Intestinal Bacteria to Form Toxic Amines, ibid. **59**:835-853, 1924; Studies on Proteinogenous Amines: XVII, On the Production of Histamine, Tyramine and Phenol in Common Laboratory Media by Certain Intestinal Micro-Organisms, ibid. **59**:855-866, 1924.

2. Koessler, K. K., and Hanke, M. T.: Studies on Proteinogenous Amines: II, A Microchemical Colorimetric Method for Estimating Imidazole Derivatives, J. Biol. Chem. **39**:497-519 (Oct.) 1919; Studies on Proteinogenous Amines: III, A Quantitative Method for Separating Histamine from Histidine, ibid. **39**:521-538 (Oct.) 1919; Studies on Proteinogenous Amines: VII, The Quantitative Colorimetric Estimation of Histidine in Protein and Protein-Containing Matter, ibid. **43**:527-542 (Sept.) 1920; Studies on Proteinogenous Amines: VIII, A Method for the Quantitative Colorimetric Estimation of Histamine in Protein and Protein-Containing Matter, ibid. **43**:543-556 (Sept.) 1920; Studies on Proteinogenous Amines: XIV, A Microchemical Colorimetric Method for Estimating Tyrosine, Tyramine and Other Phenols, ibid. **50**:235-270, 1922; Studies on Proteinogenous Amines: XV, A Quantitative Method for the Separation and Estimation of Phenols Including Phenol, o-, m-, and p-Cresol, p-Oxyphenyl-Acetic, p-Oxyphenylpropionic, and p-Oxyphenyllactic Acids, Tyrosine and Tyramine, ibid. **50**:271-288, 1922.

The various methods that have been previously used for the demonstration of bronchospasm are of two types. The first type makes use of isolated bronchial rings or strips mounted in a suitable medium so that contractions of their muscle are registered by an appropriate lever on a smoked drum. This direct method is modeled after the method of studying contractions of isolated arterial rings and has been used by Trendelenburg,[3] Titone [4] and, more recently, by Macht and Giu-Ching Ting [5] in an extensive study of the antispasmodic action of drugs.

All the other methods are indirect in principle in that contractions of the bronchi are determined from changes in the volume output of the lungs, in the lung volume, in intrapulmonary pressure, in intrathoracic pressure, in thoracic excursions or in the rate of perfusion through the pulmonary vessels. Each of these methods has yielded valuable results in the hands of the investigators who have made use of it. Thus, Brodie and Dixon [6] in their classical studies on the physiology of the lungs employed chiefly the oncometric method, applying it to a single lobe, while Jackson [7] devised a plethysmographic method involving the whole lung, and Auer and Lewis [8] encased the whole animal in a plethysmograph.

None of these methods was suitable for our purpose since we desired a method which is simple enough to be used as a routine procedure, yet which is accurate and which takes advantage of the tremendously reactive pulmonary musculature of the guinea-pig. We finally chose a method which consists of determining changes in intrathoracic pressure and which is an adaptation to the guinea-pig of a device previously used by Jackson [9] for recording changes in the movements of the lungs of dogs.

DESCRIPTION OF THE METHOD

A large guinea-pig, weighing from 500 to 800 Gm., is tied, back downward, to a table and anesthetized with ether. The trachea and external jugular vein are dissected out and prepared for ligation. The skull is exposed by making a small incision over the occiput. The bone is perforated with a sharp probe. The medulla and spinal cord are then completely destroyed with a stiff piece of wire. A cannula is promptly tied into the trachea and passive artificial respiration, with positive

3. Trendelenburg, P.: Arch. f. exper. Path. u. Pharmakol. **69**:79, 1912.
4. Titone: Pflügers Arch. f. d. ges. Physiol. **155**:77, 1914.
5. Macht, D. I., and Giu-Ching Ting: J. Pharm. & Exper. Therap. **18**: 111: 373, 1921.
6. Brodie and Dixon: J. Physiol. **29**:97, 1903.
7. Jackson, D. E.: J. Pharm. & Exper. Therap. **4**:1-59, 1912.
8. Auer, J., and Lewis, P. A.: Acute Anaphylactic Death in Guinea-Pigs, J. A. M. A. **53**:458 (Aug. 7) 1909.
9. Jackson, D. E.: Experimental Pharmacology, St. Louis, C. V. Mosby Company, p. 196.

pressure, is instituted at once. Compressed air from a tank is sent intermittently into the tracheal cannula by means of an interrupting valve that is controlled by a small electric motor (fig. 1). The inflation pressure is measured by a mercury manometer and is controlled by means of an escapement valve which is merely a piece of rubber tubing attached to the side arm of a T tube whose lumen is adjusted with a compression clamp. It is possible to regulate this clamp so that the desired amount of inflation pressure, about 5 cm. of mercury, can be

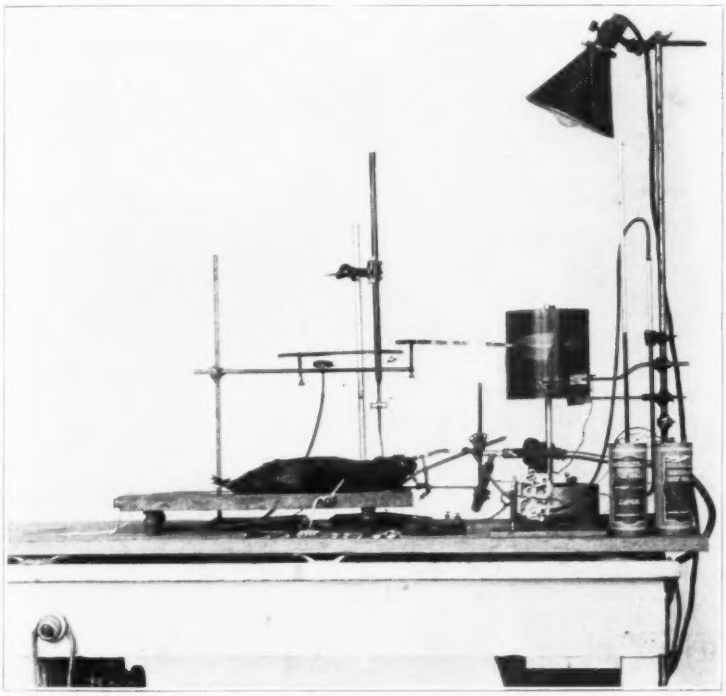

Fig. 1.—Apparatus for determining bronchospasm in the guinea-pig.

obtained. The respiration rate should be about 22 per minute. A small glass T tube is inserted just above the tracheal cannula to permit air to escape easily from the lungs during passive expiration. The heart action continues unimpaired for hours if all these operations have been properly carried out.

A small glass cannula is attached by means of a short piece of rubber tubing to a buret that is filled with physiologic sodium chloride solution. The cannula is filled to the tip with the solution and is tied

into the external jugular vein. Solutions to be tested are injected into the lumen of the rubber tubing with a hypodermic syringe and washed directly into the circulation with physiologic sodium chloride solution from the buret.

Changes in intrathoracic pressure are determined by making a communication between the chest cavity and a sensitive tambour by means of a trocar passed through the chest wall of the guinea-pig. This trocar is a number 13 gage hypodermic needle (about 3½ inches in length and $^1/_{16}$ inch in diameter). The pointed end is closed with a drop of solder without changing the shape of the point. The wall of the needle is pierced with several small openings covering a space about $^5/_8$ inch long near the center (fig. 2*a*). In order to place this trocar in the already prepared guinea-pig, a small tip of the skin over one side of the chest wall is picked up with a pair of forceps and removed with a pair of scissors. The trocar is pushed through the muscular wall and parietal pleura at about the level of the fifth or sixth intercostal space. Feeling

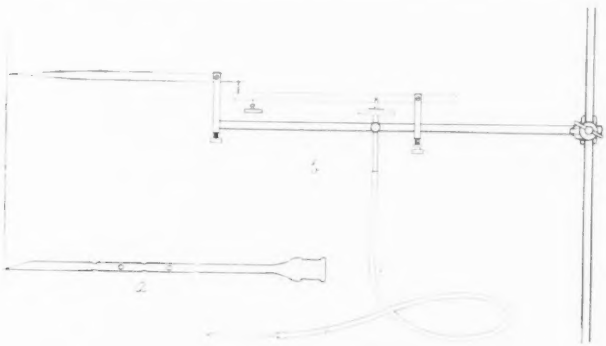

Fig. 2.—(a) Perforated trocar; (b) compound writing lever.

one's way carefully with the point of the trocar, the lungs, liver, diaphragm and heart are carefully avoided and the needle point is thrust through the pleura and muscular wall of the other side, the skin bulging over it, being cut with scissors. The open end of the trocar is attached to a soft rubber tubing of small caliber and, by means of this, to a Márey tambour. The pleural space, needle lumen (through the openings in the needle wall), rubber tubing and tambour form a closed system and any increase or decrease in pressure in the first produced by changes in lung volume is promptly transmitted to the sensitive rubber diaphragm of the tambour.

The changes in intrathoracic pressure during respiration in the guinea-pig are so slight, due to the small size of the lung, that it was found necessary to amplify the movements of the rubber diaphragm.

Fig. 3.—Effect of 0.005 mg. of histamine dichloride on the pulmonary excursion: *A*, 0.005 mg. of histamine; *B*, 0.5 mg. of atrophine; *C*, 0.5 cc. of epinephrine, 1:1,000, and *D*, 0.6 cc. of epinephrine, 1:1,000 (Nov. 26, 1923).

In order to do this the lever which rests on the diaphragm is made to actuate another lever instead of writing directly on the smoked drum. The excursion of the second lever is several times greater than that of the first, and under favorable conditions a swing of from 2 to 3 inches is produced by the movements of the guinea-pig's lungs (fig. 2 *b*).

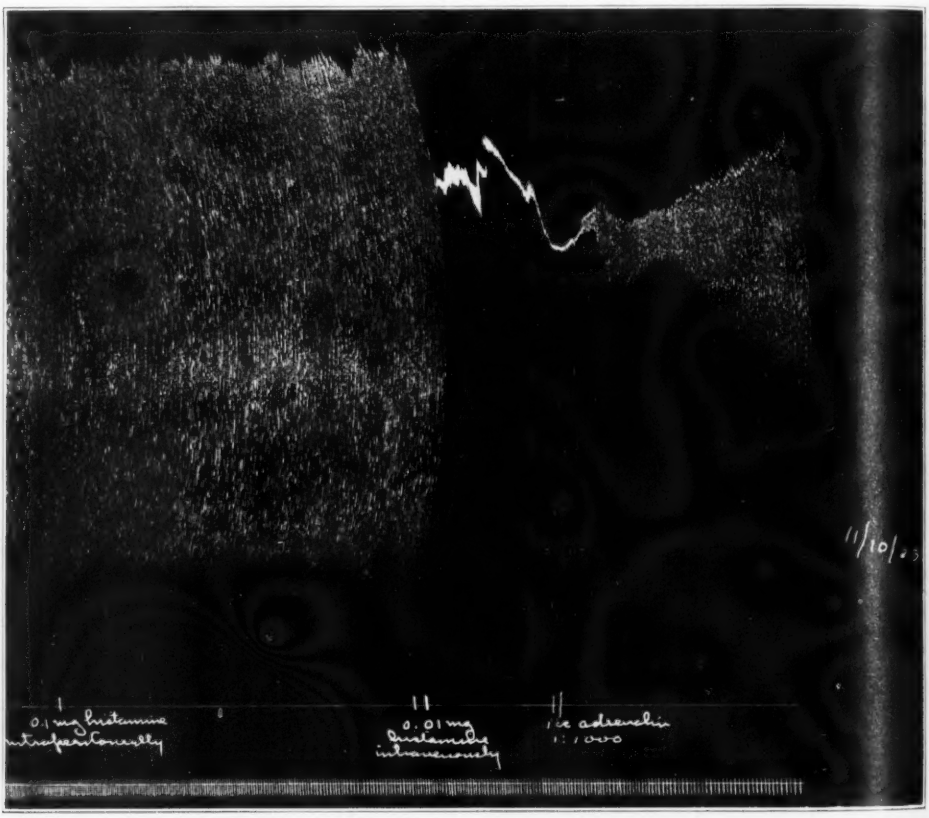

Fig. 4.—Difference of action of histamine on intraperitoneal and intravenous injection: *A*, 0.1 mg. of histamine intraperitoneally; *B*, 0.01 mg. of histamine intravenously, and *C*, 1 cc. of epinephrine, 1:1,000 (Nov. 10, 1923).

Slight changes in the movement of the lungs are plainly registered by changes in the amplitude of the curves. In reading the curves it is to be remembered that a downstroke of the lever represents an inspiration and an upstroke represents an expiration.

APPLICATION OF THE METHOD

The method was tested with a number of substances known to have a definite peripheral action on the bronchi. Probably the most active of these is histamine. Very small doses of this substance will produce death in a guinea-pig from asphyxia due to a complete blocking of the bronchi as a result of an intense contraction of the bronchial musculature. Figure 3 is a curve showing a severe bronchial spasm produced in a 700 Gm. guinea-pig with 0.005 mg. of histamine. The spasm was not

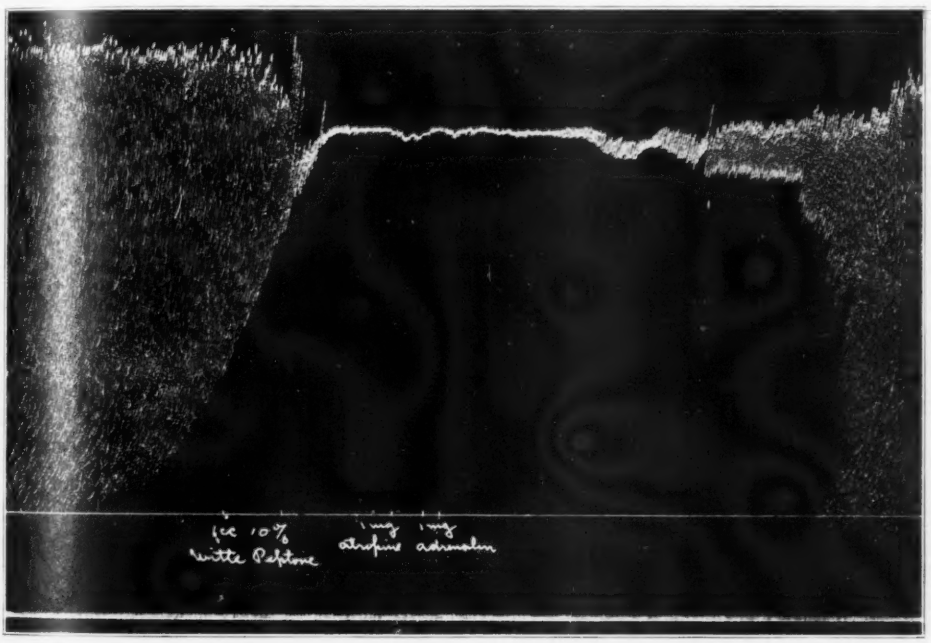

Fig. 5.—Reaction to Witte's peptone: *A*, 1 cc. of 10 per cent Witte's peptone solution; *B*, 1 mg. of atropine, and *C*, 1 mg. of epinephrine.

released with 0.5 mg. of atropine or 0.5 cc. of a 1:1,000 solution of epinephrine. It was later released with 0.6 cc. of the epinephrine.

Figure 4 shows the difference between the action of histamine injected intraperitoneally and intravenously on the bronchi. When 0.1 mg. of histamine is injected intraperitoneally into a guinea-pig, no effect can be noted, while one tenth of this dose, 0.01 mg., injected intravenously, causes the most intense bronchospasm.

Another group of substances of well-known bronchospastic action are the colloidal derivatives of protein known as peptones. Figure 5 shows the reaction of the lungs in the living pithed guinea-pig to the

Fig. 6.—Bronchospasm with Merck's peptone: *A*, 25 mg. of Merck's peptone, p_H 7.0; *B*, 4 mg. of atrophine sulphate, and *C*, 1 cc. of epinephrine, 1:1,000 (Dec. 22, 1925).

intravenous injection of 1 cc. of a 10 per cent solution of Witte peptone (100 mg.). Figure 6 represents a similar curve obtained with 25 mg. of Merck's peptone dissolved in 1 cc. of salt solution. The bronchoconstriction obtained with peptone is not entirely due to its content of histamine, for a histamine-free peptone prepared in our laboratory from fibrin [10] yields a similar pharmacologic reaction on the bronchi in the same dose as Witte peptone (fig. 7).

From the inspection of the last three curves it is again evident that the injection of atropine even in large doses was unable to arrest and

Fig. 7.—Bronchospasm with a histamine-free peptone prepared from fibrin: *A*, 0.1 Gm. of peptone from fibrin; *B*, 0.5 cc. of 1:1,000 epinephrine.

release the spasm of the bronchi, whereas 1 cc. of epinephrine (1:1,000) sufficed to accomplish this in two cases.

Many control experiments have demonstrated that this spasm, if not relieved, commonly leads to the death of the animal by asphyxia. The passive insufflation of air through the trachea is incapable of overcoming the resistance offered by the spastic stenosis of the bronchi.

10. Hanke, M. T., and Koessler, K. K.: Studies on Proteinogenous Amines: X. The Relation of Histamine to Peptone Shock, J. Biol. Chem. **43**: 567-577 (Sept.) 1920.

Para-hydroxyphenylethylamine, the amine derived by decarboxylation of tyrosine, is structurally intimately related to epinephrine. This structural relation is associated with certain similarities in physiologic behavior, as in their action on peripheral blood vessels and blood pressure.[11] The action of tyramine on the bronchi, however, is an antagonistic one to that of epinephrine, for, whereas the latter produces prompt dilatation of the contracted bronchial muscles and leads thus to disappearance of the pulmonary immobilization, tyramine usually produces bronchospasm. Whether this spasm is due to stimulation of intrapulmonary ganglions, to stimulation of sympathetic bronchoconstrictors, or to direct action on the smooth muscle, is not entirely established.[12]

In our experiments relatively large doses of tyramine hydrochloride were required to produce changes in the excursions of the lungs—from 75 to 100 mg. (fig. 8).

Since the experimental analysis of the acute anaphylactic death of the guinea-pig by Auer and Lewis,[13] Biedl and Kraus[14] and Anderson and Schultz,[15] it can be accepted as proved that death in the guinea-pig is primarily due to the asphyxia produced by a spastic stenosis of the bronchioles. This conclusion is also arrived at by Dale,[16] who in a recent investigation of anaphylaxis and anaphylatoxins, after studying changes in lung volume, says, "There is hardly room for doubt that the (bronchial) obstruction in the anaphylactic reaction is due almost entirely to plain muscle contraction."

Figure 9 represents the respiratory tracing with our method from a case of typical anaphylaxis to horse serum. The guinea-pig had been sensitized by the subcutaneous injection of 0.01 cc. of horse serum. The reaction followed an intravenous injection of 0.5 cc. of horse serum. Complete cessation of all pulmonary excursions soon took place, although the same quantity of air was delivered into the trachea as before injection. Necropsy performed while artificial respiration was continued showed the lungs maximally inflated and entirely immobile. The heart continued to beat for a short while. Another instance of horse serum anaphylaxis is shown in figure 10. The complete abolition of the pul-

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11. Barger and Dale: Arch. f. exper. Path. u. Pharmakol. **61**:113 (Sept.) 1909.

12. Bachr and Pick: Arch. f. exper. Path. u. Pharmakol. **74**:41, 1913.

13. Auer and Lewis: J. Exper. Med. **12**:15, 1910.

14. Biedl and Kraus: Handbuch der Immunitäts forschung of Kraus-Levaditi Ergänzungs, **1**:255-290, 1911.

15. Anderson, J. F., and Schultz: Proc. Soc. Exper. Biol. & Med. **7**:32, 1910.

16. Dale, H. H., and Kellaway, C. H.: Anaphylaxis and Anaphylatoxins, Philosophical Tr. **211**:273, 1922, especially pp. 304-306.

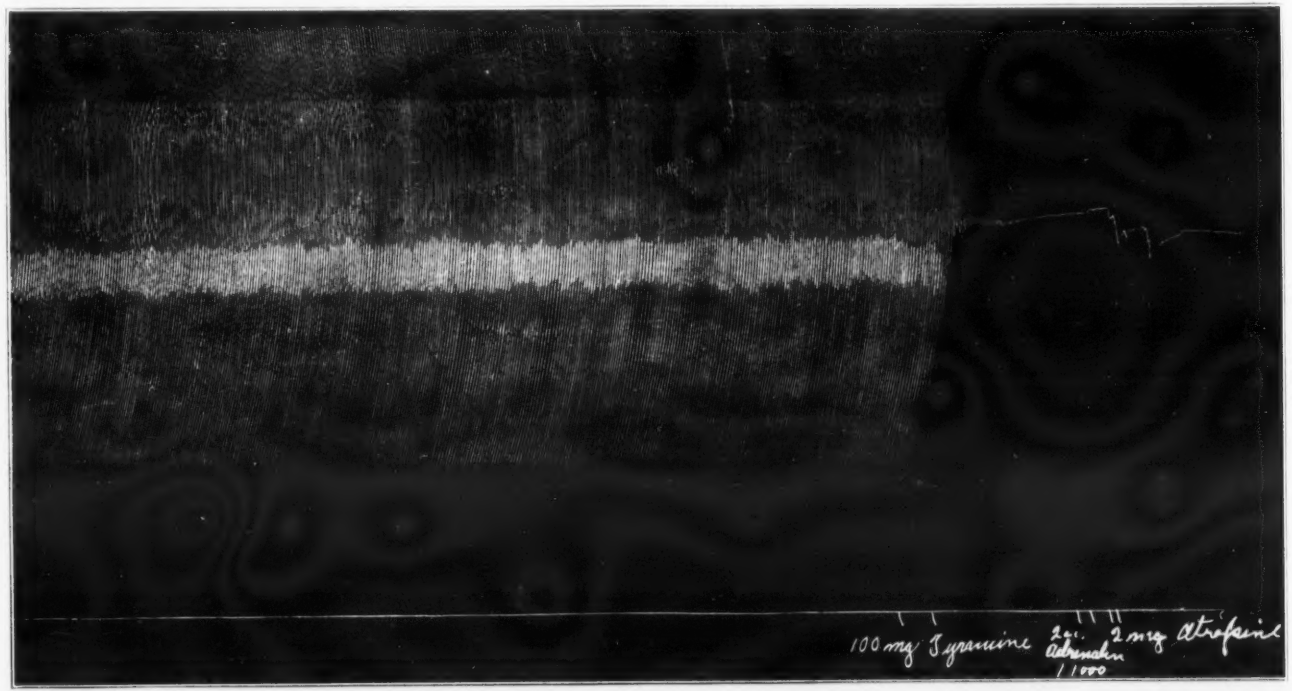

Fig. 8.—Bronchospasm with parahydroxyphenylethylamine: *A*, 100 mg. of parahydroxyphenylethylamine; *B*, 2 cc. of 1:1,000 epinephrine, and *C*, mg. of atrophine (Jan. 4, 1926).

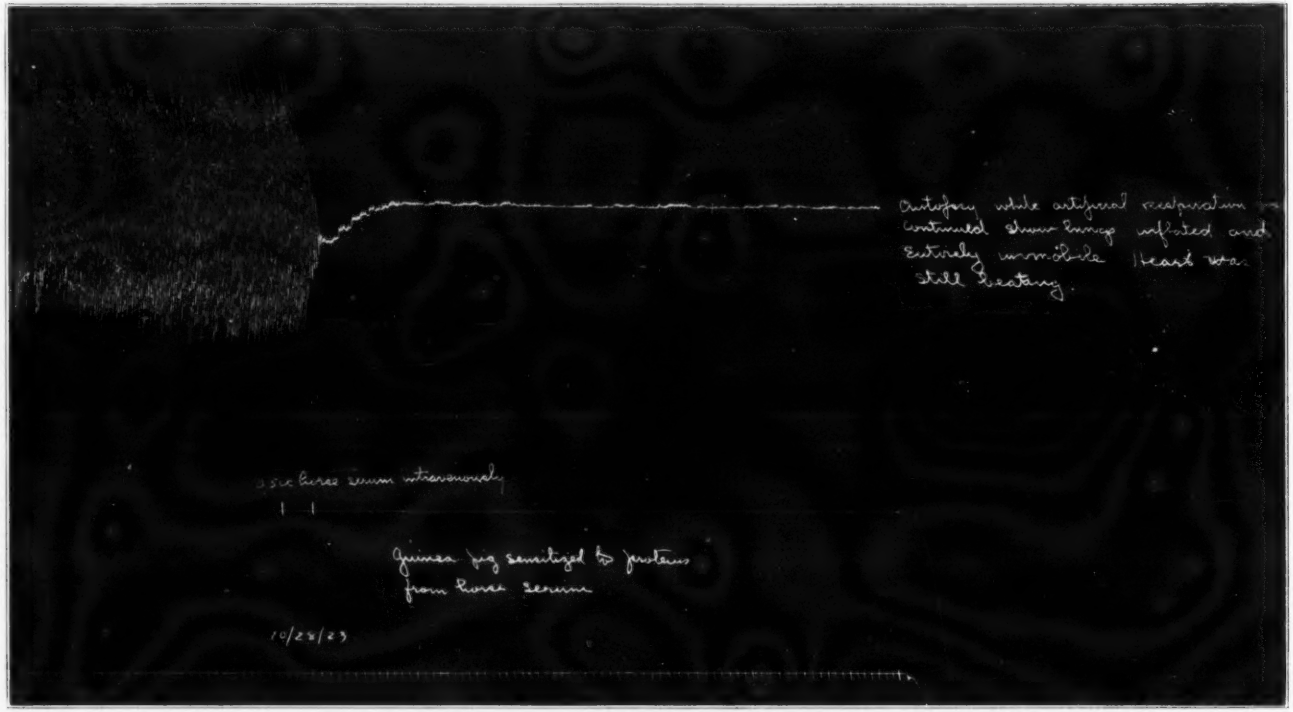

Fig. 9.—Typical anaphylaxis in guinea-pig sensitized to proteins from horse serum: *A*, 0.5 cc. of horse serum intravenously (Oct. 28, 1923). Necropsy while artificial respiration was continued showed lungs inflated and entirely immobile; the heart was still beating.

monary oscillations, which would probably have led to the death of the animal by asphyxia, was released by the intravenous injection of 0.4 mg. of atropine sulphate. Figure 11 shows a curve of the bronchial constriction in the guinea-pig in anaphylaxis to ragweed pollen.[17] Figure 12 represents an instance of passive anaphylaxis to ragweed pollen.

X 0.4 mg. atropine
Guinea pig sensitized June 12, 1923 with 0.1 cc horse serum.
Oct. 12, 1923
X 1 cc horse serum diluted 1:100 into ext. jugular

Fig. 10.—Typical horse serum anaphylaxis in guinea-pig sensitized June 12, 1923, with 0.1 cc. of horse serum: *A*, 1 cc. of horse serum diluted 1:100 injected into external jugular vein; *B*, 0.4 mg. of atropine (Oct. 12, 1923).

It has been shown that a large number of substances, crystalline as well as colloid in nature, are able to produce on first injection into the guinea-pig a group of symptoms which are practically indistinguishable from those due to true antigen-antibody anaphylaxis. Fall in blood

17. Huber, H. L., and Koessler, K. K.: The Antigenic Property of Pollens, Arch. Int. Med. **36**:751-761 (Dec.) 1925.

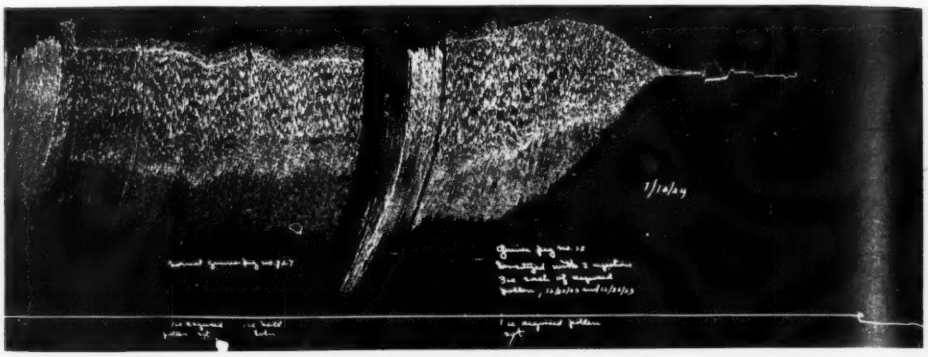

Fig. 11.—Typical anaphylaxis to ragweed pollen.

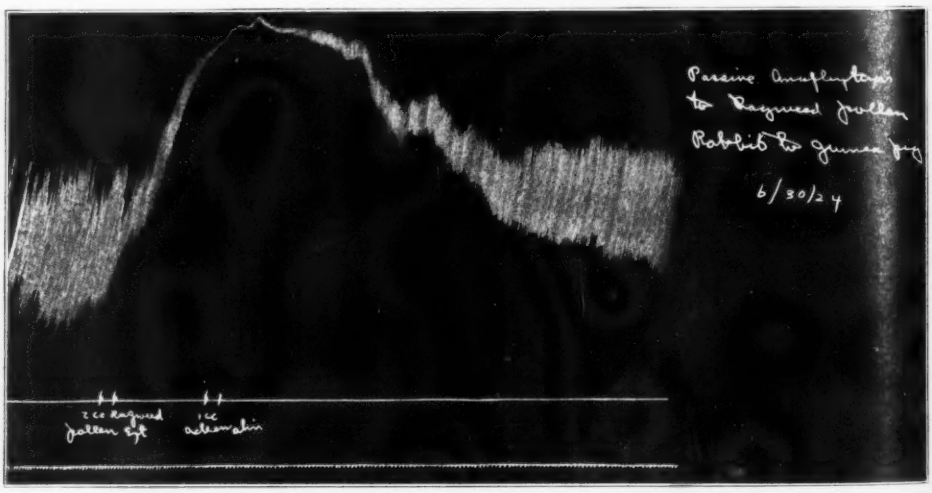

Fig. 12.—Passive anaphylaxis to ragweed pollen, rabbit to guinea-pig: *A*, 2 cc. of ragweed pollen extract, and *B*, 1 cc. of epinephrine (June 30, 1924).

pressure, leukopenia, diminished coagulability of the blood and immobilization of the lungs are all present. The important difference between these reaction phenomena and true anaphylaxis lies in the fact that these substances produce the symptoms on first injection in the normal nonsensitized animal. A thorough study of the action of such substances

Fig. 13.—Anaphylactoid reaction with toxified agar: *A*, 6 cc of toxified agar; *B*, 1 mg. of atropine.

has been made by Karsner and Hanzlik,[18] who call these phenomena "anaphylactoid reactions." The methods used by them in the study of the respiratory disorders and pulmonary changes following the injec-

18. Hanzlik, P. J., and Karsner, H. T.: J. Pharm. & Exper. Therap. **14:** 379-402 (Jan.) 1920.

tion of the various colloid substances used were perfusion of the lungs according to the method of Baehr and Pick,[12] and direct examination, microscopic and macroscopic, of the lungs. The mechanism of the action of the active agents in the production of the permanent distention of the lungs in the phase of inspiration they ascribe to occlusion of the pulmonary capillaries by thrombi of agglutinated corpuscles and plate-

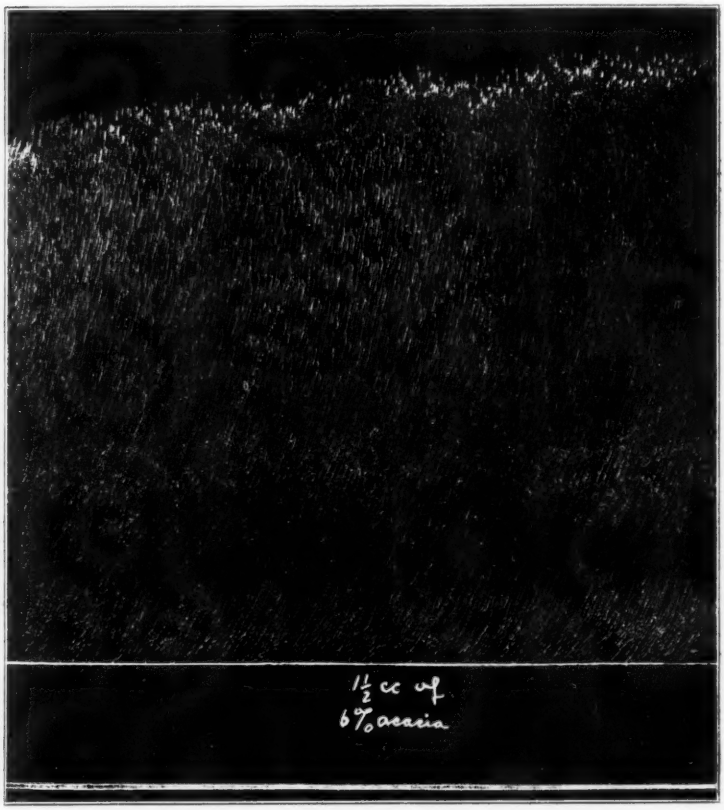

Fig. 14.—Absence of anaphylactoid reaction with acacia: *A*, 1.5 cc. of 6 per cent acacia solution.

lets, which produces bronchoconstriction. This bronchoconstriction, produces with agar solgel and other colloids, differs in origin from that produced by true anaphylaxis, peptone or histamine. It is not due to direct stimulation of the bronchial musculature or parasympathetic endings, since the effects are not antagonized by atropine or papaverine. It is suggested that embolism in the pulmonary vessels compresses the

bronchioles and thus leads to passive bronchostenosis. Our method should be an additional aid in properly evaluating the part played by true bronchoconstriction resulting in complete permanent distention of the lungs in the phase of inspiration. The pithing of the animal would exclude the central influences to which Karsner and Hanzlik ascribe certain pulmonary reactions. The results obtained with our method would seem, on the whole, to corroborate the correctness of the conclusions of Karsner and Hanzlik.

Figure 13 shows the effect of the intravenous injection of 6 cc. of toxified agar in the guinea-pig. The toxified agar was prepared according to Karsner and Hanzlik.[18] The respiratory excursions of the lungs were completely arrested with permanent distention of the organ. The intravenous injection of 1 mg. of atropine sulphate follow-

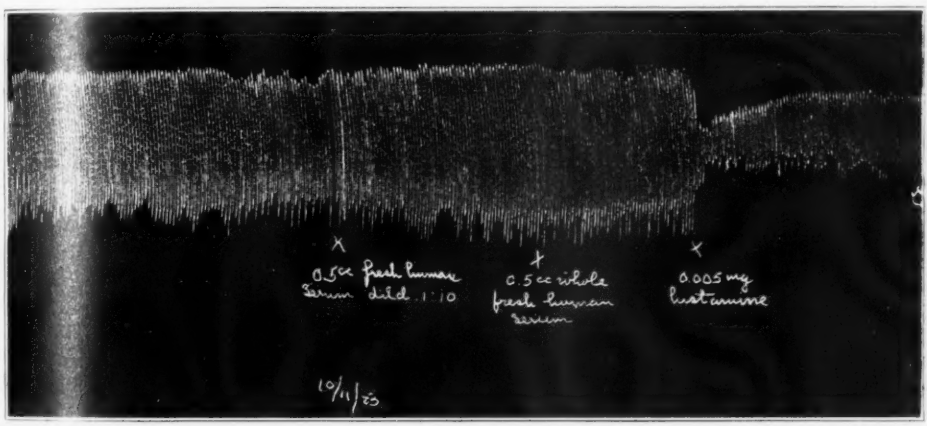

Fig. 15.—Absence of anaphylactoid reaction to fresh human serum: *A*, 0.5 cc. of fresh human serum diluted 1: 10; *B*, 0.5 cc. of whole fresh human serum, and *C*, 0.005 mg. of histamine (Oct. 11, 1923).

ing the injection of the agar was not able to relieve the respiratory distress and remove the pulmonary distention.

The effect of toxified agar in the pithed animal is proof that the seat of its action lies in the periphery; but the absence of relief by atropine seems to exclude the smooth muscle system of the bronchi as the primary seat of stimulation.

The injection of 1.5 cc. of 6 per cent acacia solution into the jugular vein of the living pithed guinea-pig was without action on the respiration of the animal (fig. 14).

No effect was obtained with 0.5 cc. of fresh human serum injected intravenously (fig. 15).

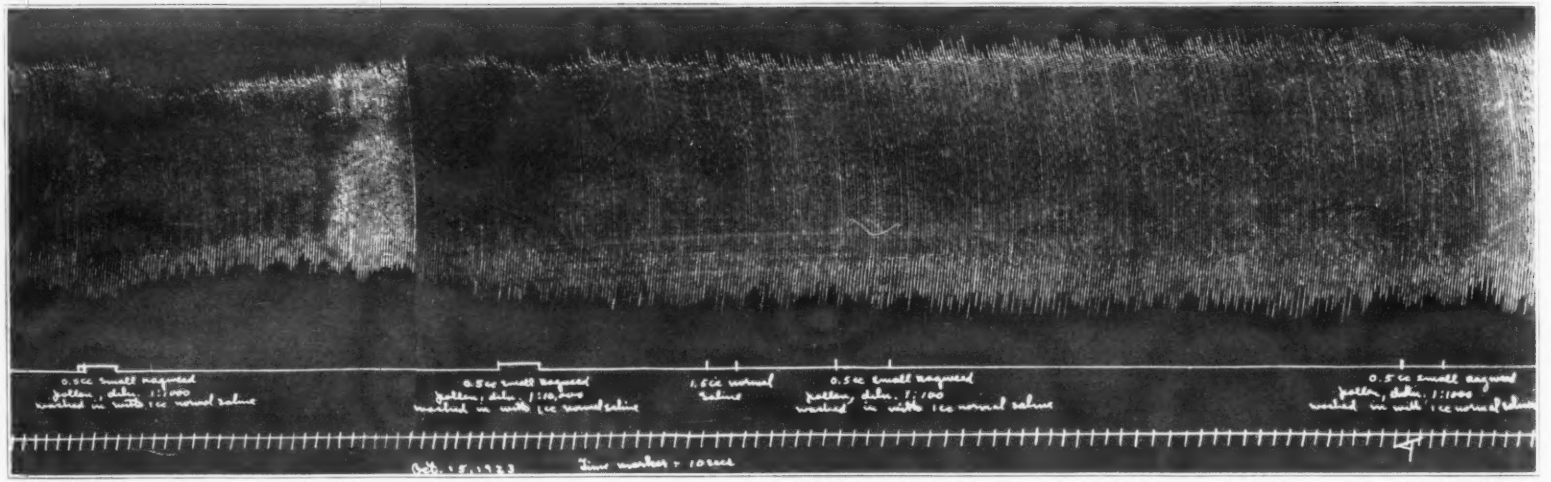
0.5 cc small ragweed
pollen, dilu. 1:1000
washed in with 1 cc normal saline
0.5 cc small ragweed
pollen, dilu. 1:10,000
washed in with 1 cc normal saline
1.5 cc normal
saline
0.5 cc small ragweed
pollen, dilu. 1:100
washed in with 1 cc normal saline
0.5 cc small ragweed
pollen, dilu. 1:1000
washed in with 1 cc normal saline
Oct. 15, 1923
Time marker = 10 secs.

Fig. 16.—Absence of anaphylactoid reaction with pollen extracts: *A*, 0.5 cc. of small ragweed pollen, diluted 1:1,000, washed in with 1 cc. of physiologic sodium chloride solution; *B*, 0.5 cc. of small ragweed pollen, diluted 1:1,000, washed in with 1 cc. of physiologic sodium chloride solution; *C*, 1.5 cc. of physiologic sodium chloride solution; *D*, 0.5 cc. of small ragweed pollen, diluted 1:100, washed in with 1 cc. of physiologic sodium chloride solution, and *E*, 0.5 cc. of small ragweed pollen, diluted 1:1,000, washed in with 1 cc. of physiologic sodium chloride solution (Oct. 15, 1923). Time marker, 10 seconds.

In a recent article on the antigenic action of pollen by Koessler and Huber [17] it was shown that typical anaphylaxis can be produced with pollen extracts in guinea-pigs under proper experimental conditions. A necessary preliminary to this investigation was the proof that the intravenous injection of the pollen extracts used does not give rise to changes in pulmonary movements in the nonsensitized animal, especially since Karsner and Hanzlik, working with an entirely different technic, reported respiratory disturbances which they ascribe to hemorrhages and conglutination thrombi.

Figure 16 shows that even 0.5 cc. of a 1 : 100 dilution of ragweed pollen extract does not produce the slightest change in the pulmonary excursions of a pithed guinea-pig.

Figure 11 demonstrates that bronchoconstriction is brought about promptly under the same experimental conditions in the sensitized animal.

CONCLUSIONS

In the method for graphic demonstration of bronchospasm in the guinea-pig described, the procedure is simple and can be carried out as a routine procedure in any laboratory.

A number of substances (histamine, para-hydroxyphenylethylamine and various peptones) of known bronchospastic activity were tested with this technic.

The method has proved itself a valuable aid in the experimental analysis and diagnosis of anaphylaxis and anaphylactoid phenomena.

DEMONSTRATION OF ARTERIAL CONSTRICTION IN VITRO

A NEW METHOD *

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The most characteristic and constant feature of the anaphylactic reaction is the contraction of smooth muscle. It is this manifestation which accounts for the sudden and dramatic clinical picture that is ordinarily produced in anaphylaxis, especially in the common laboratory animals. According to the animal used, either the contraction of the smooth muscle of one system of organs is more massive than that of another, or, because of different anatomic relations, more profound symptoms are produced. Whether or not there is a participation to some extent of all the smooth muscle tissue in the body, or whether there is a selective action for certain groups, cannot be decided at present, either because of the nonappearance of clinical symptoms which may identify the participation of certain organs, or because of the lack of suitable methods to demonstrate the in vitro response of the smooth muscle in all organs.

Because of certain similarities that exist between the anaphylactic reaction and the action of certain amines, e. g., histamine and tyramine hydrochloride, the same problems as to the localization of action on smooth muscle likewise apply to the amine action as well as to anaphylaxis. It can be demonstrated by the Dale [1] technic that the uterine and intestinal smooth muscle of the guinea-pig is contracted in anaphylaxis and by the application of histamine and tyramine hydrochloride. The active participation of the smooth muscle of the bronchi in the anaphylactic and amine reactions can be demonstrated by the technic of Koessler and Lewis [2]; but the great bed of smooth muscle, the vascular system, has not been studied with the same exactness with which the uterine, intestinal and bronchial musculature have been investigated. It is true that the effect of anaphylaxis and of the amines on the blood pressure is known, but changes in blood pressure are the result of a complicated reaction and do not permit an analysis of the reaction of vascular smooth

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1. Dale and Laidlow: J. Pharm. & Exper. Therap. **4**:75, 1912.

2. Koessler, K. K., and Lewis, J. H.: The Determination of Bronchospasm in the Guinea-Pig, Arch. Int. Med. **39**:163, 1927.

muscle per se. For example, the reaction of an isolated artery to histamine would lead one to expect a pressor reaction in vivo instead of the marked depressor effect that is characteristic of this substance.

Studies of the action of substances directly on the arteries have usually been made by the perfusion method, which consists of forcing through the blood vessels of an organ a fluid that contains the material to be studied. The effect is determined by the changes noted in the rate of outflow. This procedure has given valuable information to many investigators and is one of the standard methods in physiologic work. It has, however, certain drawbacks, and results obtained by the method show occasional inconsistencies. Macht [3] has pointed out that errors arise from the variation in the method of inserting the cannulas and from changes that occur in the parenchyma of the organ during perfusion. It is well known that the tissues of organs under the artificial conditions of perfusion often swell to such an extent that circulation through the blood vessels is completely blocked.

Myer [4] devised a technic of studying the reaction of isolated blood vessels which is comparable to that used by Schultz [5] and Dale [1] for the isolated uterus. A cross-section of a large artery from an animal such as the cow is divided so as to form a strip and is mounted in the Dale apparatus. In order to use smaller arteries, a series of small rings of arterial wall is united by means of loops of thread, thus making a chain of them. When mounted in the Dale apparatus a constriction of the circular fibers of each ring will produce a shortening of the whole chain. Voegtlin and Macht [6] improved this technic and described the procedure of applying a stretching load to the chain of arterial rings which overcomes the marked initial tonus of an isolated artery. The stretched chain is provided with a smaller lifting load, which remains attached during the experiment. Our results with this method were not satisfactory, first, because it is a rather tedious operation to tie together four or more small rings of an artery such as a pig's carotid; second, because the threads used to tie the rings together either stretch or cut the arterial rings when subjected to the weights necessary to overcome the primary tonus, or unless great care is taken in tying them, often pull apart when weighted. There is a marked distortion of the rings and an unequal distribution of the force to all the muscle fibers of the preparation as a result of the stretching. Again, it is difficult to get the required relation between stretching load and lifting load. It is different for each preparation, and if it is not properly adjusted, the line produced by the writing lever is not straight; it shows either a

3. Macht: J. Pharm. & Exper. Therap. **6**:13, 1914.
4. Myer: Ztschr. f. Biol. **48**:352, 1906.
5. Schultz: Hygiene Laboratory Bulletin, no. 80, 1912.
6. Voegtlin and Macht: J. Pharm. & Exper. Therap. **5**:79, 1913.

gradual return of the original tonus or a continuous stretching. The response of this preparation to such active vasoconstrictors as epinephrine and histamine is sluggish and protracted, and is not comparable to the activity shown by the uterus strip or segments of intestines. The uncertainty of this method has been pointed out previously by Müller.[7]

After many efforts to improve this technic and make it adaptable to our purpose, we finally devised another method in which the arterial preparation is a continuous band of tissue representing a considerable length of artery, so prepared that the circular musculature is made

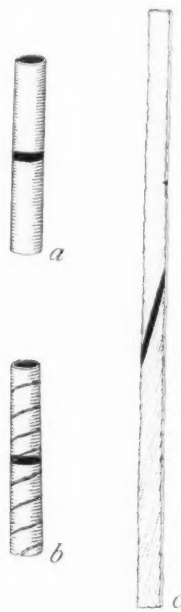

Fig. 1.—Steps in the preparation of an artery to demonstrate vasoconstriction. The heavy black marks represent the direction of the circular muscle fibers before and after the artery is cut and unrolled; the length of *c* is that obtained by starting with the length of *a*.

to lie perpendicularly; when it contracts, a shortening of the preparation occurs. An artery, such as the pig's carotid, is trimmed of all loose connective tissue. One blade of a small pair of scissors is inserted into one end and a winding spiral incision is made the whole length of the artery. The scissors are slanted so that the incision at one place is about one-eighth inch (0.3 cm.) below the incision above it. When the incision is completed, the preparation resembles the paper winding that is

7. Müller, F.: Arch. f. Anat. u. Physiol., 1906, supp. vol., p. 411.

removed from a wax pencil. It is now unfurled by running the whole ribbon between the fingers and slightly stretching. This process causes the circular muscle fibers to run in the direction of the length of the ribbon (fig. 1). A thread is now tied on both ends. A length about 4 inches (10 cm.) of the prepared artery is used, although a longer or shorter one may serve. We usually employ a double strip as it seems to give a sharper reaction and to last longer. The strip is now mounted in the chamber of the Dale apparatus, which contains Locke's solution through which oxygen is bubbled. The lever is attached so that it is in the position of maximal contraction. A 20 Gm. weight is applied to the lever for about ten minutes, after which a 2 Gm. weight is attached. The lever is adjusted and the weight shifted until there is no movement up or down, and a straight line is written on a slowly moving drum. The preparation is now ready for use. Substances that produce vaso-

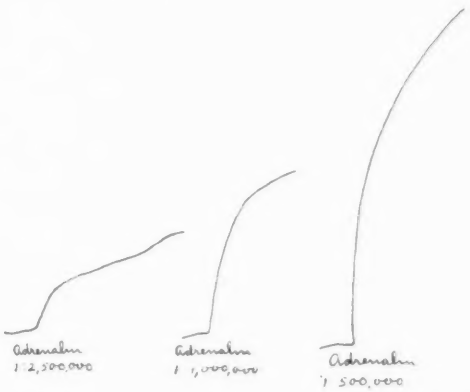

Fig. 2.—Arterial constriction in vitro due to epinephrine.

constriction will cause, when added to the liquid in the chamber, a sudden sharp movement of the lever, the height of which depends on the concentration of the active substance. If the contraction has been large, the original position of the lever is recovered slowly. Our procedure is to note the height to which the curve goes, then wash the preparation with three changes of Locke's solution, after which the 20 Gm. weight is applied for about five minutes. This can be done without removing the 2 Gm. weight. The preparation is now ready for use again. This can be repeated ten or fifteen times before it gives out. The first sign of fatigue of the muscle is a failure to rise to its normal level after removal of the 20 Gm. weight.

It is our custom to obtain from twelve to fifteen pigs' carotids from the stockyards and place them immediately in a bottle of Locke's solution containing a small amount of pig's blood. If the bottle is kept cold and

the Locke's solution aerated every day with oxygen, the arteries will remain usable for at least one week. When in a good condition the artery has a small lumen and appears as a rigid pink tube when trimmed of excessive connective tissue. As the arteries get old they become white, the lumen flattens out and they are flabby. When in such condition, they should be discarded.

This method is sensitive to vasoconstrictors. Figure 2 shows curves obtained with various dilutions of epinephrine hydrochloride; figure 3,

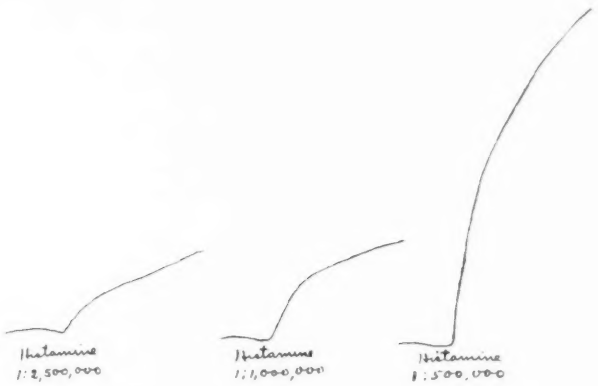

Fig. 3.—Arterial constriction in vitro due to histamine.

similar curves obtained with various dilutions of histamine. But even concentrations of 1 : 10,000,000 of epinephrine hydrochloride, as well as of histamine, give definite and clear cut contractions. Tyramine hydrochloride is less active, the limits of its activity being from about 1 : 500,000 to 1 : 1,000,000.

The interest in guanidine and its methyl derivatives and their possible relation to arterial hypertension has recently been revived through the work of Major.[8] Rather high concentrations of these substances, however, are required to show any effect on the isolated arteries in vitro, a reaction that corresponds well with their behavior in the living animal (fig. 4).

The constancy and uniformity of the response is remarkable. The same concentration of vasoconstrictor substance will give the same amount of contraction with the same preparation time after time.

8. Major, R. H., and Stephenson, W.: The Effect of Methyl Guanidine on the Blood Pressure, Bull. Johns Hopkins Hosp. **35**:140 (May) 1924; Further Observations on the Elevation in Blood Pressure Produced by Guanidine Compounds, ibid. **35**:186 (June) 1924; The Excretion of Guanidine Bases in Two Cases of Arterial Hypertension with Reduction in Blood Pressure, ibid. **36**:357 (May) 1925.

We have used the method extensively to study the vasocontrictor action of bacterial poisons, a problem that has been the subject of work for years in this laboratory. Bacteria are grown in liquid mediums of various compositions and under different conditions. The Berkefeld filtrates of these cultures are tested in a routine manner as to their action on the isolated artery. Some of these filtrates are active in their effect, and their activity can in some instances be shown, by chemical analysis, to be due to histamine and tyramine hydrochloride, but in many

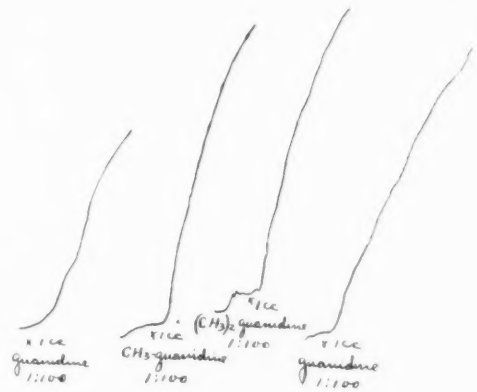

Fig. 4.—Arterial constriction in vitro due to guanidine, methyl and dimethyl guanidine.

other cases the active substance is chemically not identified as yet. A discussion of these studies forms the subject matter of the next article in the series.[9]

9. Koessler, K. K.; Lewis, J. H., and Walker, J. A.: Pharmacodynamic Actions of Bacterial Poisons, Arch. Int. Med. **39**:188, 1927.

PHARMACODYNAMIC ACTIONS OF BACTERIAL POISONS *

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Probably no expression is more frequently used by the clinician and pathologic physiologists to describe certain phenomena during acute and chronic diseases due to bacterial infection than the term "toxemia" or "the toxic action of the micro-organism." Such expressions denote our general tacit agreement that the processes by which pathogenic micro-organisms cause disease consist in the last instance in the chemical action of such poisons on certain organs or organ systems. Yet our real knowledge regarding such action is disproportional to the frequency of the usage of the word toxemia, and this statement holds true nearly as much for the few specific secretory products of the bacteria, the true toxins in the immunologic sense of the word, as for the unspecific poisons that are formed by the interaction of micro-organisms and tissue cells.

During the last ten years our group of workers in the Otho S. A. Sprague Memorial Institute has concentrated its efforts on one phase of this problem, which was concerned with the chemical study of certain poisons which are formed when micro-organisms grow in a medium containing protein and amino-acids. Quantitative chemical methods were devised that permitted the isolation and determination of these poisonous substances, which are, for the most part, chemically well characterized amines. It could be shown that micro-organisms which form histamine or tyramine are normal inhabitants of the human intestinal tract, and the presence of these toxic amines in the normal intestinal content was proved by actual chemical isolation. This work further proved that the catabolism of amino-acids in the intestinal tract proceeds normally, not only by deamination, but also by decarboxylation. But more recent investigation showed that this faculty of decarboxylation leading to amine production is not restricted to intestinal bacteria. The mixture of common pyogenic micro-organisms contained in the bronchial secretion, in sputum, in various foci of infection, such as the tonsils, and in inflammatory exudates, such as empyema fluids, is able to form large quantities of these poisonous amines, such as histamine and tyramine, in vitro.

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The object of the study reported here was not to determine the production and mode of action of the chemically well defined crystalline substances like histamine and tyramine, but to follow up the evidence obtained early in this work that other poisons, as yet chemically undefined, are formed by bacteria under our experimental conditions—poisons which might have a definite pharmacologic activity.

PROCEDURE

The micro-organisms were all grown in 200 cc. of a peptone-blood broth, which contained 1 per cent peptone, 2 per cent glycerine, 5 per cent whole sheep blood, and 0.1 per cent histidine or tyrosine. This medium is inoculated with a single strain of bacteria or with the natural mixture of micro-organisms contained in sputum or other body fluids. After an incubation period of two weeks, 25 cc. of the culture is filtered through a Berkefeld filter and set aside in sterile tubes for p_H determination and pharmacologic tests. The remaining liquid is heated on the water bath to kill the bacteria and coagulate the proteins.

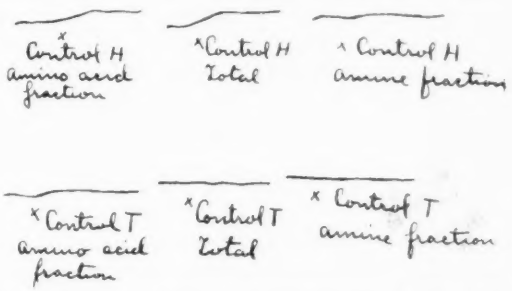

Fig. 1.—Control tests: Absence of vasoconstrictor effect of the uninoculated medium fractioned according to the usual procedure.

Complete coagulation can sometimes be obtained only after adding a few drops of glacial acetic acid. The mixture is then filtered and the filtrate evaporated on the water bath in a glass dish. The residue is transferred to a graduated cylinder and diluted exactly to 25 cc. When this filtrate could not be analyzed immediately, it was preserved by adding 0.5 cc. of concentrated sulphuric acid. The acid has no effect either on the chemical determinations or on the pharmacologic activity after proper neutralizing. Ten cubic centimeters of this liquid is then treated with 3 Gm. of sodium hydroxide and extracted six times with amyl alcohol to remove amines. The amyl alcohol fraction is extracted with sulphuric acid. The acid is neutralized with sodium hydroxide and evaporated to dryness on the water bath. The residue is dissolved in water and diluted to 25 cc. The residue remaining after extraction with amyl alcohol is treated with 7 cc. of 37 per cent hydrochloric acid solution, evaporated to dryness, dissolved in water and diluted to 25 cc.

By these manipulations the original blood broth culture is divided into four fractions: (1) filtrate filtered through a Berkefeld, but not chemically treated; (2) the concentrated filtrate obtained after removal of the proteins; (3) the amine fraction, which is soluble in amyl alcohol, and (4) the amino-acid fraction, which remains after extraction with amyl alcohol.

The amine fraction, which contains all the amines, was used for the chemical determination of these substances. This and the other fractions after exact neutralization were used in various ways to assay their pharmacologic activity.

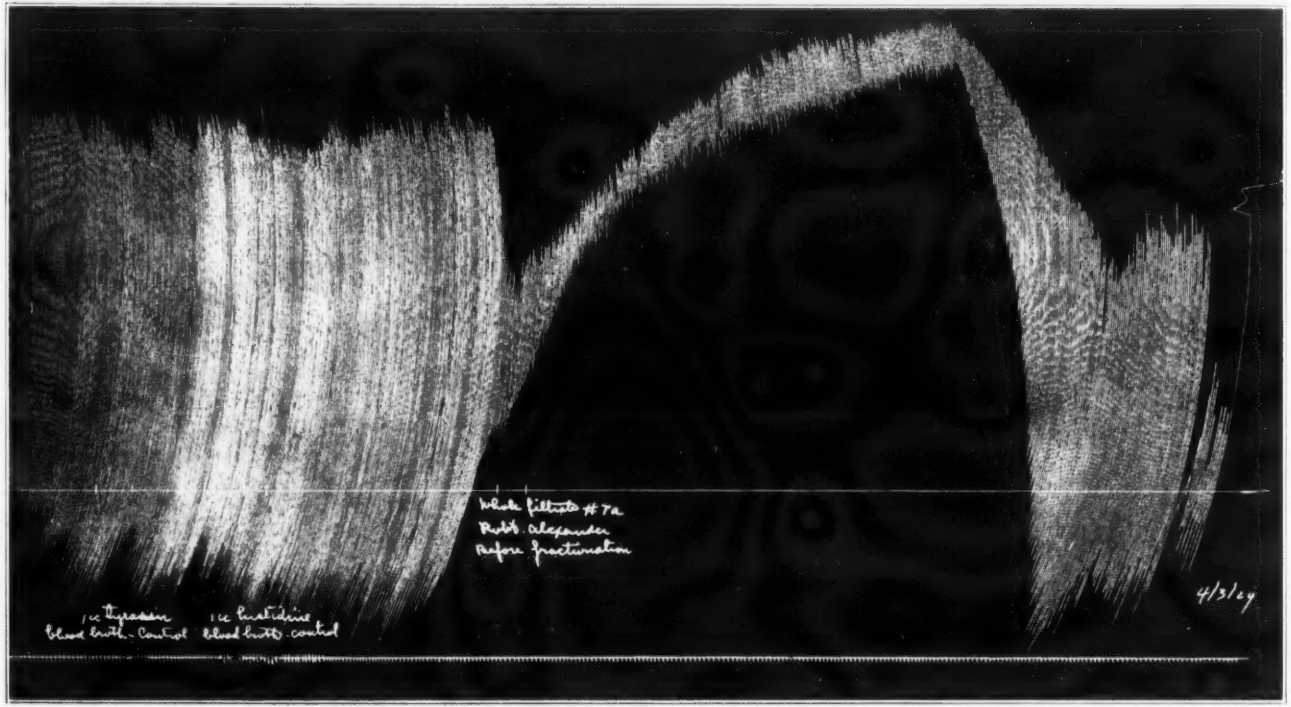

Fig. 2.—*A*, control experiment with tyrosine and histidine glycerol blood broth medium without inoculation; *B*, whole filtrate from a histidine glycerol blood broth medium that had been inoculated with sputum 7 R. A., showing a marked bronchiolar spasm.

The pharmacologic tests were based on the well-known action of amines, typified in histamine and tyramine on smooth muscle tissue. A method which utilizes the smooth muscle of arteries and another one which depends on the reaction of bronchial musculature, both of which are described in the preceding articles, were used as routine procedures in testing these fractions.

CONTROLS

The methods were well controlled in that the tests were carried out with the Berkefeld filtrate from the standard uninoculated blood-glycerol broth containing histidine or tyrosine. These sterile mediums were fractioned as described above for the cultures. The results obtained with these control tests were uniformly negative (figs. 1 and 2). Further controls were obtained throughout the routine testing of the bacterial filtrates, in that numerous filtrates gave negative results.

In several hundred experiments on arteries and bronchi the amino-acid fraction proved to be free from physiologic activity as to vasoconstrictor and bronchospastic action. The physiologic activity, if present, resides in the Berkefeld filtrate, the total concentrated filtrate and the amine fraction. Since the unheated Berkefeld filtrate showed the same behavior as it did after removal of the proteins, through acetic acid and heat coagulation, pharmacologic tests were subsequently restricted to the total concentrated filtrate, the amine fraction and the amino-acid fraction. The physiologic activity of the amine fraction always corresponds qualitatively to that of the total filtrate, but varies in intensity. Equal amounts of the amine fraction are usually more active than the total filtrates.

In studies previously made on the production of proteinogenous amines,[1] especially histamine and tyramine, by intestinal micro-organisms, isolated specific strains of bacteria were originally used, but so many of these proved to be negative as regards decarboxylation of the amino-acid, and the resulting formation of the corresponding amine, that it was decided to work with the natural mixture of micro-organisms contained in human feces. In this way it could be shown that the intestinal content of man contains, in about 70 per cent of all cases examined, organisms that are able to form histamine or tyramine. Pure strains that were able to convert the amino-acids into the corresponding amines were then isolated from some of these stools. This method of using a naturally occurring mixture of micro-organisms shortened our procedure and gave valuable preliminary information. It was therefore adopted in the present study of the amine production by organism of the respiratory tract.

The mediums were first inoculated with the mixture of bacteria occurring in the expectorated sputum. A small amount of freshly obtained sputum was introduced into a test tube containing 5 cc. of sterile broth, which was vigorously rotated between the hands to wash off surface contamination. Then three loopfuls of this mixture were transferred into another tube containing 5 cc. of broth, and after vigorous shaking to obtain a good suspension, three loopfuls were transferred to two flasks of medium, one containing tyrosine and one histidine. After two weeks' incubation the cultures were fractionated as described above and the fractions studied chemically and pharmacologically.

RESULTS

It soon became evident, in contradistinction to the results obtained with intestinal organisms, that in the majority of instances both tyramine and histamine were completely absent; yet the filtrates possessed very

1. Literature references are given in the preceding articles: Lewis, J. H., and Koessler, K. K.: Demonstration of Arterial Constriction in Vitro: A New Method, Arch. Int. Med. **39**:182, 1927. Koessler, K. K., and Lewis, J. H.: The Determination of Bronchospasm in the Guinea-Pig: Applications of the Method, ibid. **39**:163, 1927.

powerful pharmacologic amine activity, as evidenced by the action of the whole filtrate and the amine fraction on the bronchial and arterial musculature.

This observation was made on the first sputum from a pneumonia patient examined. It had been shown in our studies on the formation of proteinogenous amines by decarboxylation that the production of these poisonous substances was invariably associated with a change in reaction of the medium toward the acid side. Only in a distinctly acid medium are histamine and tyramine formed. The p_H of the first pneumonia sputum examined (Thomas Healy), after inoculation and fourteen days' incubation in histidine blood-glycerine-broth, was 7.8. This in itself was strongly suggestive evidence that histamine or tyramine had not been formed, which was proved to be the case by chemical analysis. The alkaline reaction of the medium does not preclude the formation of substances of amine structure; it would suggest, however, that they are not formed by decarboxylation (triamines?). In 0.5 cc. amounts of the total test solution and amine fractions a most intensive bronchoconstriction and vasoconstriction were obtained. The amino-acid fraction, on the other hand, was pharmacologically completely inert, showing that the active principle is completely soluble in amyl alcohol, another item of evidence as to its amine nature (fig. 3).

The inoculation of a tyrosine blood broth medium with a sample of the same sputum made on the same day did not yield a trace of tyramine; neither was any pharmacologic action obtained with any of the three fractions.

The behavior of a different sample of sputum from another patient with pneumonia (Ben Green) was entirely at variance from that of the first one. In the histidine medium, 62 per cent of the histidine was converted into histamine, and because of this amine 0.5 cc. of the amine fraction given intravenously immediately killed with bronchospasm a guinea-pig of 600 Gm. (fig. 4). An intense arterial constriction was obtained with the total and amine fractions. The same sputum when inoculated into the tyrosine medium yielded 45.6 per cent tyramine. The fractions of this culture also produced a fatal bronchospasm and an intense arteriospasm. The action on the bronchi in this instance, however, cannot be attributed entirely to tyramine, for this substance exerts such an action only in a much higher dose than was present in 1 cc. of the amine fraction. The arterial constriction could have been due to the tyramine.

Sputum sample 4 was obtained from patient M., suffering from a severe bronchitis and tonsillitis; in the histidine medium no decarboxylation (p_H 7.6) occurred and not a trace of histamine was formed. But the total test solution and amine fraction contained a bronchospastic poison of such intensity that 1 cc. killed a 700 Gm. guinea-pig in apnea

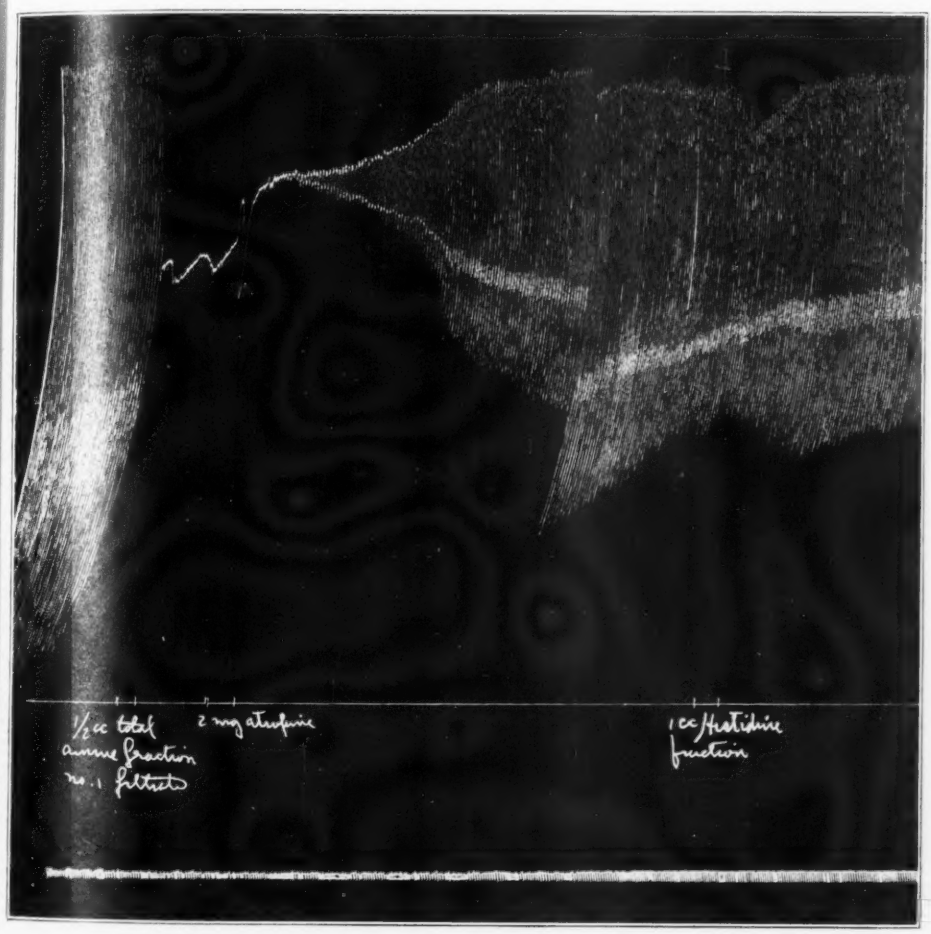

Fig. 3.—Amine fraction of sputum 1 T. H.: histamine and tyramine were absent; the bronchiolar spasm was caused by some substance of unknown constitution, possibly an amine.

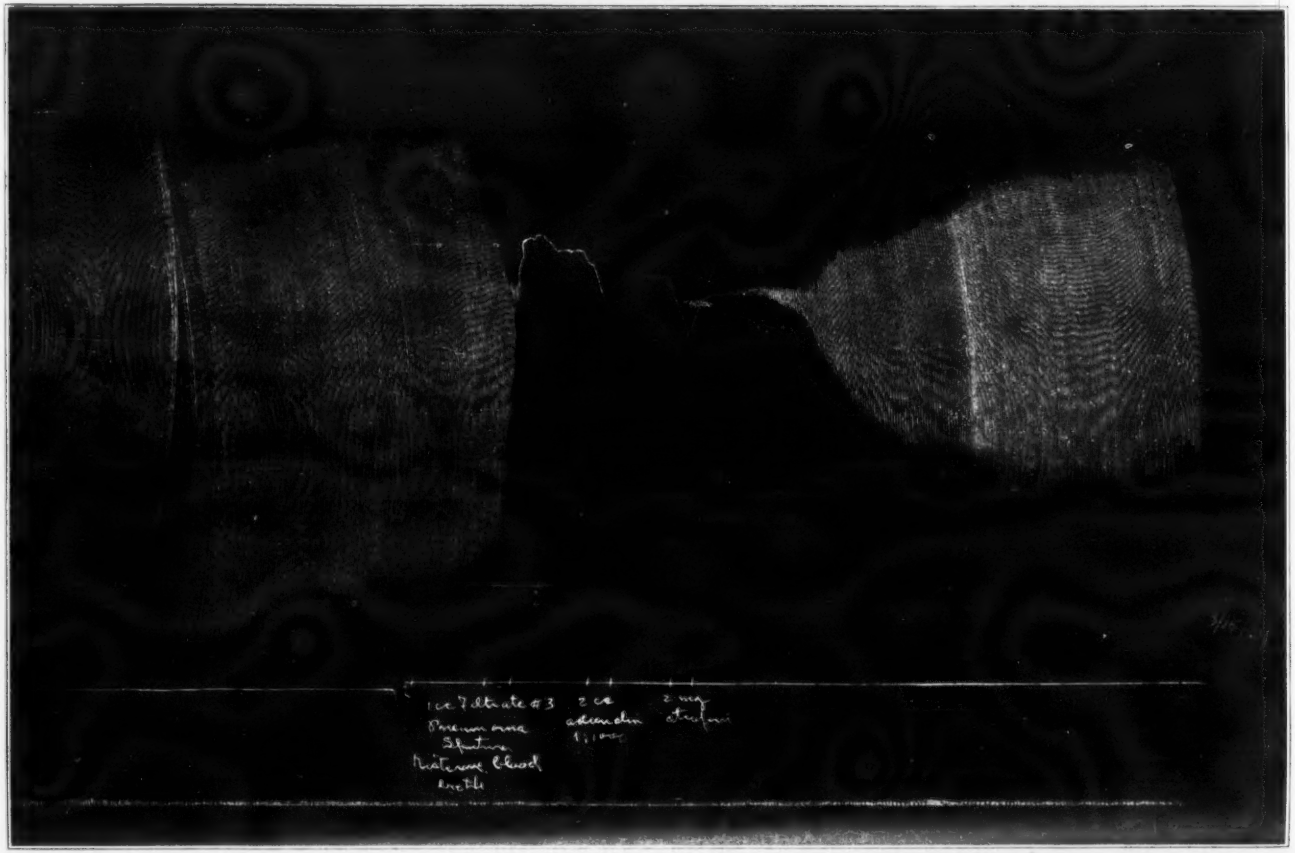

Fig. 4.—Total filtrate of sputum 3 P. J.: The fatal bronchiolar spasm was due to the histamine which had been produced by this mixture of micro-organisms.

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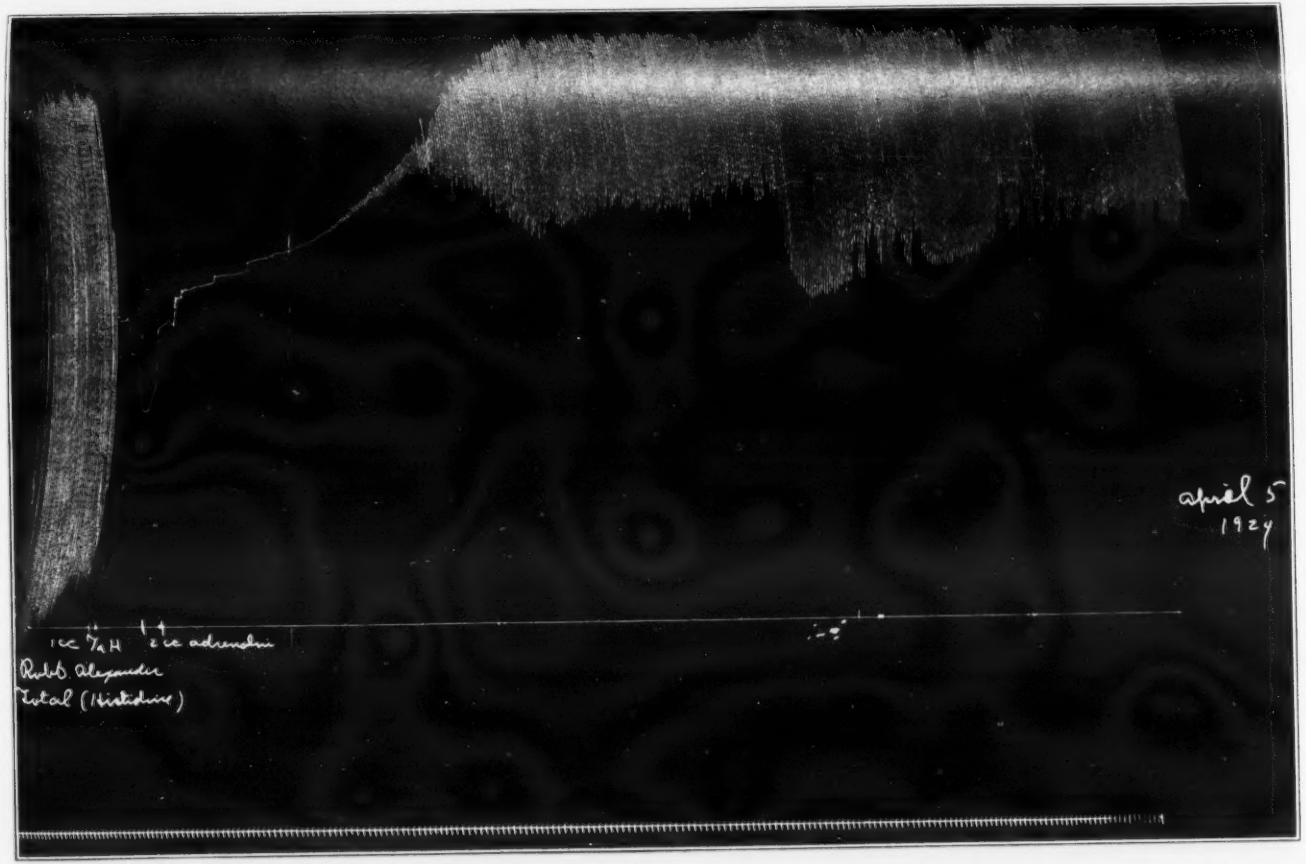


Fig. 5.—Total filtrate of sputum 7 R. A.: The bronchiolar spasm was due to poison of unknown constitution; histamine was absent

(fig. 5); also, a marked arterial constriction was obtained with the same fractions (fig. 6). The amino-acid fraction again proved completely without pharmacodynamic effect. The sputum inoculated on the same day and in the same quantity into the tyrosine medium led to the formation of 125 mg. of tyramine, which represents 60.2 per cent conversion. The p_H of the medium after fourteen days' incubation was 5.6. An intense degree of arterial and bronchial spasm was produced in the pharmacologic tests.

In sputum 5, taken from patient P. S., suffering from lobar pneumonia, no histamine was formed after two weeks' incubation of the

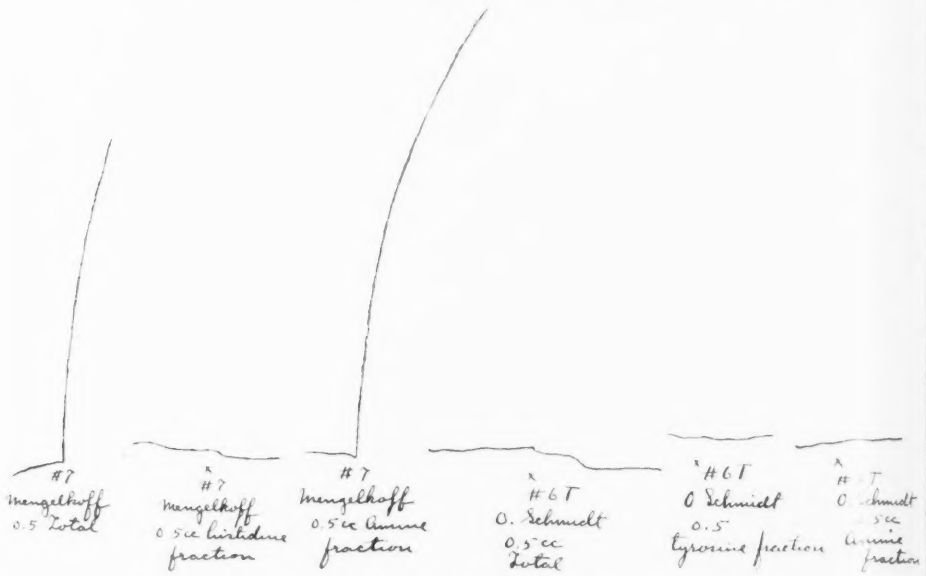


Fig. 6.—Arterial constriction obtained with fractions from medium that had been inoculated with sputum 4 M. K.: The absence of contraction with the amino-acid fraction should be noted. Pus sample 6 O. S. did not contain arteriospastic substances.

inoculated histidine-blood-broth-glycerine medium. The pharmacologic test showed a pronounced bronchial and vascular constriction. In the tyrosine medium decarboxylation of tyrosine with 98 per cent conversion into tyramine took place. Both pharmacologic tests proved strongly positive (fig. 7).

The pus from two postpneumonic empyemas, containing many pneumococci, proved entirely negative, while the incubation of the purulent sputum of a lung abscess led to the formation of a poison which gave intense bronchospasm, but was without effect on the arteries. This

selective action of the poison, while not the rule, has been encountered several times. Most frequently the poison which causes bronchiolar constriction leads also to vascular constriction in vitro. In some instances, however, the activity is restricted to only one of the smooth muscle fiber systems; intense vasoconstriction may result from a poison

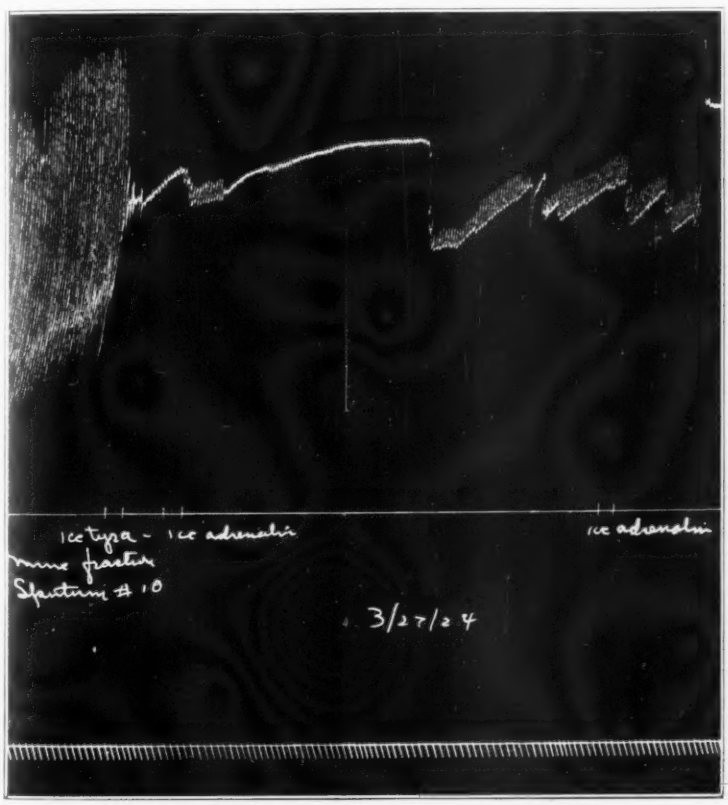


Fig. 7.—Amine fraction of sputum 10 S. S.: This fraction contained tyramine, but the bronchiolar spasm obtained cannot have been caused by this amine.

contained in the amine fraction, which is without effect on the pulmonary excursions and the bronchi (fig. 8).

In this manner the sputum of many patients suffering from various respiratory diseases was inoculated into the blood-glycerin broth containing histidine and tyramine, and the fractions obtained examined for physiologic activity. In addition, empyema fluid and pus from lung abscesses were treated in a similar manner. It was found that there

were by far many more instances in which not a trace of histamine or tyramine had been formed than instances in which these amines could five led to the production of histamine derived from the histimine con- be demonstrated. Yet in the absence of any one of these two amines we frequently—in about 70 per cent of all cases examined—found substances which acted distinctly on the bronchi and the arteries.

Table 1 contains a list of the sputums, empyema fluids and other five led to the production of histamine derived from the histidine con-

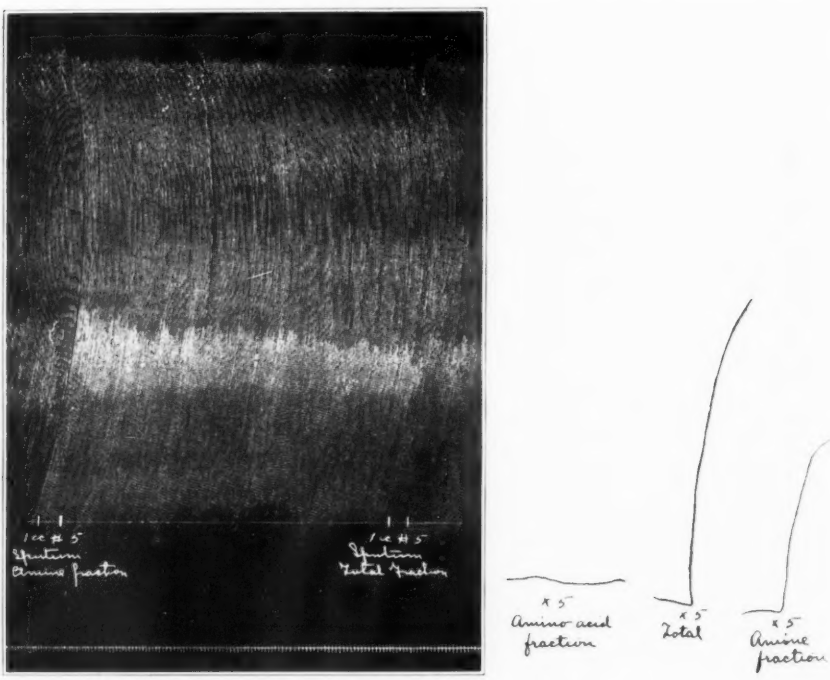


Fig. 8.—Fractions from sputum 5 P. S.: Bronchospastic substances were absent and arteriospastic substances were present. The absence of constrictor substances in the amino-acid fraction should be noted.

tained in the medium. Five produced tyramine by decarboxylation of the tyrosine added to the medium.

Of the thirty-seven specimens, five produced histamine; five produced tyramine; fifteen formed bronchospastic substances which were neither histamine nor tyramine; eighteen formed arteriospastic substances, neither histamine nor tyramine; one produced both histamine and tyramine, and nineteen produced both bronchospastic and vasoconstrictor substances.

TABLE 1.—*Production of Bronchospastic and Arteriospastic Substances by the Mixture of Micro-Organisms Contained in Sputums and in Purulent Exudates*

Case	Sputum from Case	Diagnosis	Reaction After Incubation: Blood Broth Medium p_H	Blood Broth Medium Plus Histidine p_H	Blood Broth Medium Plus Tyrosine p_H	Histidine Recovered per Cent	Histidine Converted into Histamine per Cent	Tyrosine Recovered per Cent	Tyrosine Converted into Tyramine per Cent	Bronchospasm	Arterial Spasm
1	1. T. H.	Pneumonia	...	7.8	...	10	None	.....	.....	4+	4+
2			...	...	6.6	..	.....	None	None	None	None
3	2. B. G.	Pneumonia	...	5.6	...	75	62	.....	.....	4+	4+
4			...	...	5.8	..	.....	None	45.6	4+	4+
5	3. P. J.	Empyema	...	5.8	...	53	None	.....	.....	None	4+
6			...	...	7.8	..	.....	Present	None	None	None
7	4. M. K.	Bronchitis	...	7.6	...	90	None	.....	.....	4+	4+
8			...	...	5.6	..	.....	Present	64.75	3+	4+
9	5. P. S.	Pneumonia	...	5.2	...	50	None	.....	.....	3+	4+
10			...	...	5.2	..	.....	None	98.0	4+	3+
11	7. R. A.	Pulmonary tuberculosis	...	7.3	...	35	None	.....	.....	4+	4+
12			...	...	7.1	..	.....	None	72.0	4+	4+
13	9. Os.	Lung abscess	...	7.6	...	50	None	.....	.....	4+	None
14			...	...	8.0	..	.....	None	None	2+	None
15	10. S. S.	Bronchiectasis	...	7.4	...	70	None	.....	.....	None	3+
16			...	...	5.2	..	.....	None	None	None	2+
17	11. C. D.	Bronchitis	...	6.4	...	30	None	.....	.....	3+	1+
18			...	...	7.1	..	.....	75	None	...	None
19	12. R. G.	Pneumonia	...	8.0	...	50	None	.....	.....	None	4+
20			...	...	8.2	..	.....	Present	None	None	None
21	13. S. U.	Pulmonary tuberculosis	...	7.2	...	40	None	.....	.....	4+	4+
22			...	...	7.2	..	.....	Present	None	4+	3+
23	14. S.	Bronchiectasis	...	5.8	...	110	34	.....	.....	...	4+
24			...	...	7.7	..	.....	None	None	4+	4+
25	15. C. C.	Asthma	...	7.3	...	92	None	.....	.....	4+	4+
26			...	...	7.6	..	.....	Present	None	4+	4+
27			7.6	...	...	40	None	.....	.....	4+	3+
28	16. T. J. W.	Pneumonia	...	7.6	...	90	None	.....	.....	4+	4+
29			...	...	6.0	..	.....	Present	None	4+	4+
30			7.8	...	...	40	None	.....	.....	4+	4+
31	17. C. S.	Pneumonia	...	5.4	...	110	None	.....	.....	4+	4+
32			...	...	6.0	..	.....	Present	None	4+	4+
33			5.0	...	...	52	None	.....	.....	4+	4+
34	18. M. T.	Pneumonia	...	7.6	...	45	45	.....	.....	4+	4+
35			...	...	6.2	..	.....	Present	None	4+	4+
36			6.0	...	...	15	None	.....	.....	...	...
37	22. W. P.	Bronchiectasis	...	7.6	...	65	None	.....	.....	None	1+
38			...	...	6.6	..	.....	None	None	None	None
39	23. J. C.	Bronchiectasis	...	6.6	...	95	33.5	.....	.....	4+	4+
40			...	...	7.3	..	.....	Present	None	4+	4+
41	24. A. L.	Pneumonia	...	6.4	...	45	None	.....	.....	None	None
42			...	...	7.4	..	.....	Present	None	None	None
43	25. R. S.	Bronchiectasis	...	5.6	...	100	None	.....	.....	1+	None
44			...	...	6.4	..	.....	None	None	None	None
45	26. C. D.	Pneumonia	...	5.6	...	110	None	.....	.....	None	None
46			...	...	6.0	..	.....	.....	None	None	None
47	27. E. S.	Pneumonia	...	7.6	...	43	None	.....	.....	None	None
48			...	...	5.4	..	.....	None	None	None	None
49	28. O. W.	Pneumonia	...	7.8	...	84	None	.....	.....	None	3+
50			..	...	7.4	..	.....	.....	None	None	None
51	29. W. D.	Pneumonia	...	7.6	...	23	None	.....	.....	None	None
52			...	...	7.6	..	.....	.....	None	None	None
53	30. G. L.	Tuberculosis	...	7.1	...	73	None	.....	.....	4+	4+
54			...	...	6.2	..	.....	Present	None	1+	None
55	31. G. B.	Pneumonia	...	7.6	...	87	None	.....	.....	None	2+
56			...	...	5.2	..	.....	Present	None	4+	4+
57	32. M. J.	Pulmonary tuberculosis	...	7.3	...	28	None	.....	.....	1+	4+
58			...	...	6.6	..	.....	Present	None	None	None

TABLE 1.—*Production of Bronchospastic and Arteriospastic Substances by the Mixture of Micro-Organisms Contained in Sputums and in Purulent Exudates—Continued*

Case	Sputum from Case	Diagnosis	Reaction After Incubation: Blood Broth Medium p_H	Blood Broth Medium Plus Histidine p_H	Blood Broth Medium Plus Tyrosine p_H	Histidine Recovered per Cent	Histidine Converted into Histamine per Cent	Tyrosine Recovered per Cent	Tyrosine Converted into Tyramine per Cent	Bronchospasm	Arterial Spasm
59	33. P. H.	Pulmonary tuberculosis	...	7.6	...	20	None	.....	.....	None	None
60			...	...	6.6	..	.....	Present	None	None	None
61	34.	Pneumonia	...	6.6	...	..	.....	.....	.....	None	1+
62			...	...	7.4	..	.....	.....	.....	None	None
63	35.	Bronchitis	...	6.0	...	..	.....	.....	.....	4+	4+
64			...	...	6.6	..	.....	.....	.....	None	None
65	36.	Pneumonia	...	6.4	...	..	.....	.....	.....	None	None
66			...	...	6.8	..	.....	.....	.....	4+	...
67	37.	Influenza	...	5.8	...	..	.....	.....	.....	None	None
68			...	...	6.4	..	.....	.....	.....	None	None
69	38. B. V.	Pneumonia	...	7.6	...	16	None	.....	.....	...	...
70			...	...	7.3	..	.....	None	None	4+	4+
71	39. C. G.	Bronchial abscess	...	5.0	...	..	.....	.....	.....	4+	3+
72			...	...	7.6	..	.....	.....	.....	None	None
	Pus Samples										
1	6. O. S.	Empyema	...	6.6	...	70	None	.....	.....	None	None
2			...	...	7.6	..	.....	Present	None	None	None
3	8. B. F.	Empyema	...	7.1	...	60	None	.....	.....	None	None
4			...	...	7.3	..	None	.....	.....	None	None
5	Thomas	Maxillary sinus	...	6.0	...	..	73.8	.....	.....	4+	4+
6			...	...	6.4	..	.....	.....	72.2	4+	4+

Thus, in all, twenty (54 per cent) produced bronchospastic substances, and twenty-six (70 per cent) formed arteriospastic substances.

While the study of the pharmacodynamic action of such mixtures of micro-organisms as are contained in pathologic bronchial secretions and in other inflammatory exudates is of great interest, it is evident that the results obtained at once invited the extension of this study to the metabolic products formed by isolated pure strains of micro-organisms, to ascertain which ones were responsible for the production of the poisonous substances. Therefore, the pathologic fluids that produced active filtrates were cultured and as many organisms isolated in pure culture as possible. These pure strains were then inoculated, in counted numbers, into the standard mediums. From then on the procedure was the same as with the whole mixture.

Sputum 7 was studied first in this way. The specimen came from a patient who had an extensive tuberculosis of both lungs, with small cavities. A secondary infection was associated with the tuberculous infection and a gram strain of the sputum showed many gram-positive and gram-negative organisms.

The total sputum in the histidine as well as tyrosine blood broth medium had produced bronchospastic and arterioconstrictor poisons, the

first of such intensity that the guinea-pig was killed in both instances on intravenous injection of 1 cc. of the total concentrated fraction. This filtrate contained not a trace of histamine, but 72 per cent conversion of tyrosine to tyramine had taken place. In all, eleven organisms were isolated from his sputum. One, designated as organism 3, was a small gram-positive streptococcus. In the histidine-glycerin-blood broth this organism formed a poison, not histamine, which caused a marked

Fig. 9.—Organism 21 isolated from sputum 7 R. A.: The bronchospasm was due to histamine.

arterial constriction in vitro, but no bronchiolar contraction in the living pithed guinea-pig. The same coccus grown for the same period (fourteen days) in the tyrosine medium formed a poison, not tyramine, which produced a marked bronchoconstriction and arterioconstriction.

Another organism, 21, isolated from this sputum, was a gram-negative bacillus resembling *Bacillus subtilis* in certain morphologic and chemical characteristics. It formed an unidentified poison which caused intense bronchoconstriction (fig. 9) as well as vasoconstriction. A stock culture as well as a freshly isolated typical strain of *B. subtilis* caused

only arterial constriction, but was without effect on the pulmonary excursions.

Organism 11, a gram-negative bacillus, caused marked bronchiolar spasm (fig. 10) but was without effect on the arterial musculature in vitro. Organism 42, a long gram-negative bacillus, yielded fractions in the culture mediums which were without effect in pharmacologic tests.

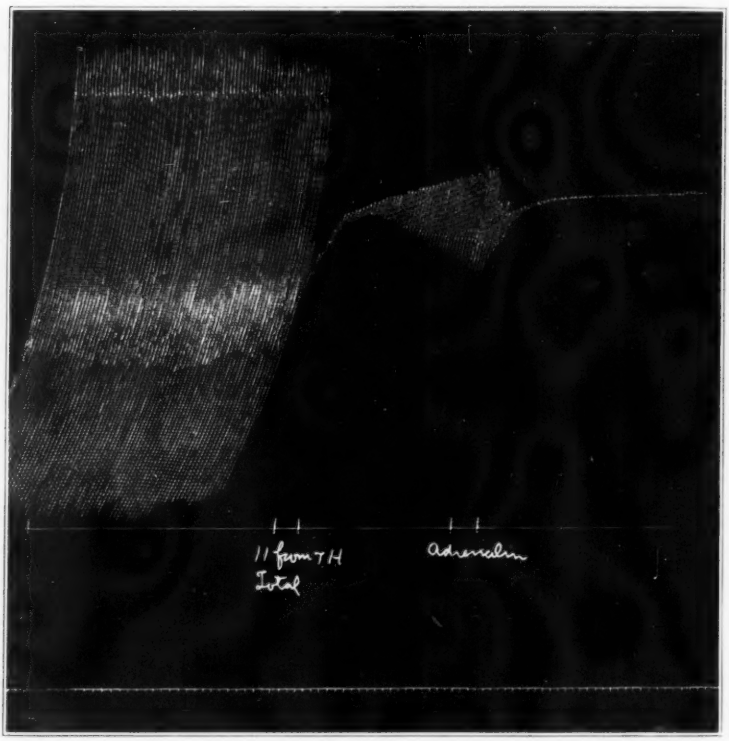


Fig. 10.—Organism 11 from sputum 7 R. A.: The bronchospasm was due to substances of unknown chemical constitution; histamine was absent.

A gram-positive yeast, organism 38, formed no active poison. Organism 9, a gram-positive coccus, different in morphologic and cultural characteristics from organism 3, formed, like this coccus, a substance active on the arteries but inactive on the bronchi.

Organism 10, another gram-positive coccus, formed 98 per cent tyramine in the tyrosine medium and showed intense arteriocontraction but only weak bronchoconstriction.

A specimen of a purulent exudate from a subacute inflammation of a maxillary sinus (patient S. Thomas) yielded *Staphylococcus aureus*

and a streptococcus. Both organisms formed a substance in the glycerin-blood broth histidine medium which caused arterial constriction, but which was without effect on lung volume. The results obtained with the purulent exudates of these various sources and the pure strains isolated therefrom are given in table 2.

TABLE 2.—*Production of Bronchospastic and Arteriospastic Substances by Pure Strains of Micro-Organisms*

Number of Micro-organism	Source	Classification	Reaction After Incubation: Blood Broth Medium Plus Histidine pu	Reaction After Incubation: Blood Broth Medium Plus Tyrosine pu	Histidine Recovered, per Cent	Histidine Converted into Histamine, per Cent	Tyrosine Recovered, per Cent	Tyrosine Converted into Tyramine, per Cent	Bronchospasm	Arterial Spasm
1	Sputum 7 R. A....	Gram-negative coccus	...	5.0	...	.....	Present	None	None	None
3	Sputum 7 R. A....	Gram-negative coccus	...	5.0	...	.....	Present	None	3+	3+
9	Sputum 7 R. A....	Gram-positive coccus	...	5.0	...	.....	60	None	None	4+
10	Sputum 7 R. A....	Gram-positive coccus	...	5.0	...	.....	.....	98	1+	4+
3H	Sputum 7 R. A....	Gram-positive coccus	5.2	...	132	None	.....	.....	None	3+
11H	Sputum 7 R. A....	Gram-negative bacillus	7.2	...	122	None	.....	.....	4+	None
21H	Sputum 7 R. A....	Gram-positive bacillus	5.6	...	132	75.7	.....	.....	4+	4+
21H	Sputum 7 R. A....	Six months later	7.6	...	90	None	.....	.....	4+	4+
28H	Sputum 7 R. A....	Gram-positive bacillus and yeast	5.8	...	140	None	.....	.....	None	None
32H	Sputum 7 R. A....	Gram-negative bacillus	7.8	...	132	None	.....	.....	None	None
1	Sputum 21 F. N...	Gram-positive coccus	7.0	...	110	None	.....	.....	None	1+
1	Pus (Thomas)....	.....	6.0	...	...	.....	.....	.....	None	4+
2	Pus (Thomas)....	.....	4.8	...	...	.....	.....	.....	None	4+
339	.....	B. paratyphosus A	5.2	...	...	None	.....	.....	4+	4+
339	.....	B. paratyphosus A	...	5.2	...	.....	Present	None	3+	4+
212	.....	B. paratyphosus A	5.2	...	...	None	.....	.....	None	4+
212	.....	B. paratyphosus A	...	6.0	...	.....	Present	None	None	4+
251	.....	B. paratyphosus B	5.2	...	...	None	.....	.....	None	4+
251	.....	B. paratyphosus B	...	5.0	...	.....	Present	None	4+	4+
404	.....	B. paratyphosus B	6.0	...	...	None	.....	.....	None	4+
404	....	B. paratyphosus B	...	6.4	...	.....	Present	None	4+	None
Barmore		B. typhosus...	5.8	...	...	None	.....	.....	None	None
Barmore		B. typhosus...	...	5.8	...	.....	Present	None	None	4+
E. B.		B. typhosus...	5.0	...	...	None	.....	.....	4+	1+
E. B.		B. typhosus...	...	6.2	...	.....	Present	None	4+	3+
Hopkins		B. typhosus...	5.4	...	...	None	.....	.....	4+	4+
Hopkins		B. typhosus...	...	5.6	...	.....	Present	None	None	None
		Dysentery Shiga	6.0	...	...	None	.....	.....	None	4+
		Dysentery Shiga	...	6.0	...	.....	Present	None	None	1+
		Dysentery Flexner	7.6	...	...	None	.....	.....	None	1+
		Dysentery Flexner	...	7.6	...	.....	Present	None	None	1+
C. C. H.		B. coli.....	6.0	...	...	31	.....	.....	4+	4+
		B. coli.....	...	6.6	...	.....	Present	None	None	None

A number of other pathogenic organisms in pure culture were studied, some of which were recently isolated, while others were stock cultures.

All of eight strains of *Bacillus typhosus* and *Bacillus paratyphosus* yielded filtrates that caused intense arterial constriction, but only three of this number produced substances that caused bronchospasm.

A study of the colon group, in the stricter sense, emphasizes anew that the production of poisonous substances pharmacologically active is

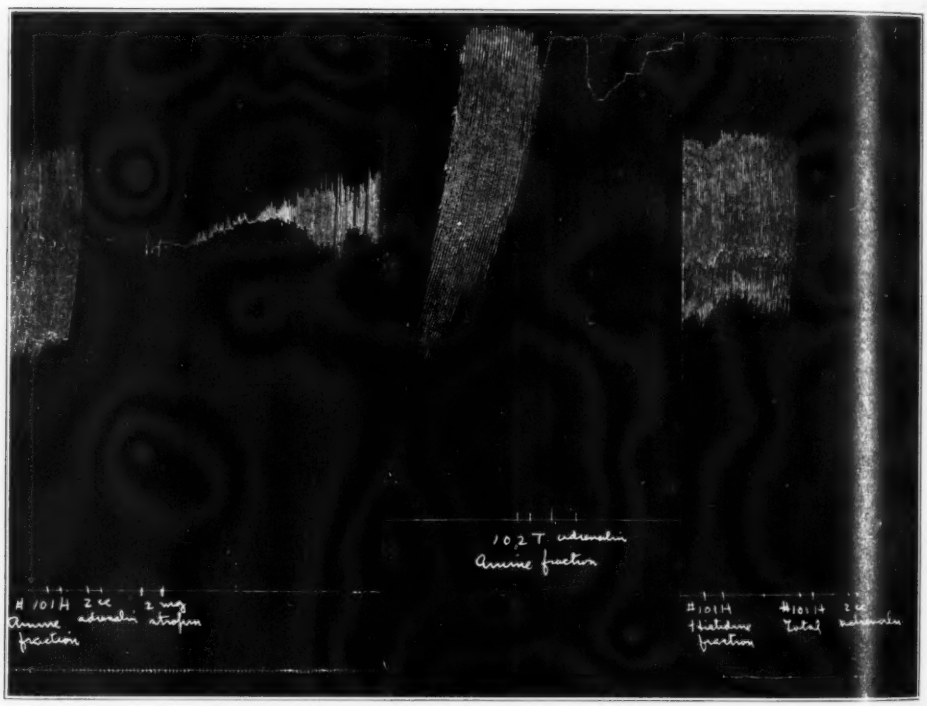


Fig. 11.—Bronchospasm obtained with streptococci.

associated with certain strains of a bacterial species, which by their other cultural and chemical behavior cannot be distinguished from other strains of the same species. It is a peculiar property with which the bacteriologist and immunologist have been acquainted for years. For example, among the organisms that form a true toxin in the immunologic sense, as the diphtheria, tetanus, dysentery and Welch bacillus, certain strains can be found which do not possess this faculty of forming a toxin, or at least to only a slight degree. Yet these strains differ in no other respect except in their ability to form toxin.

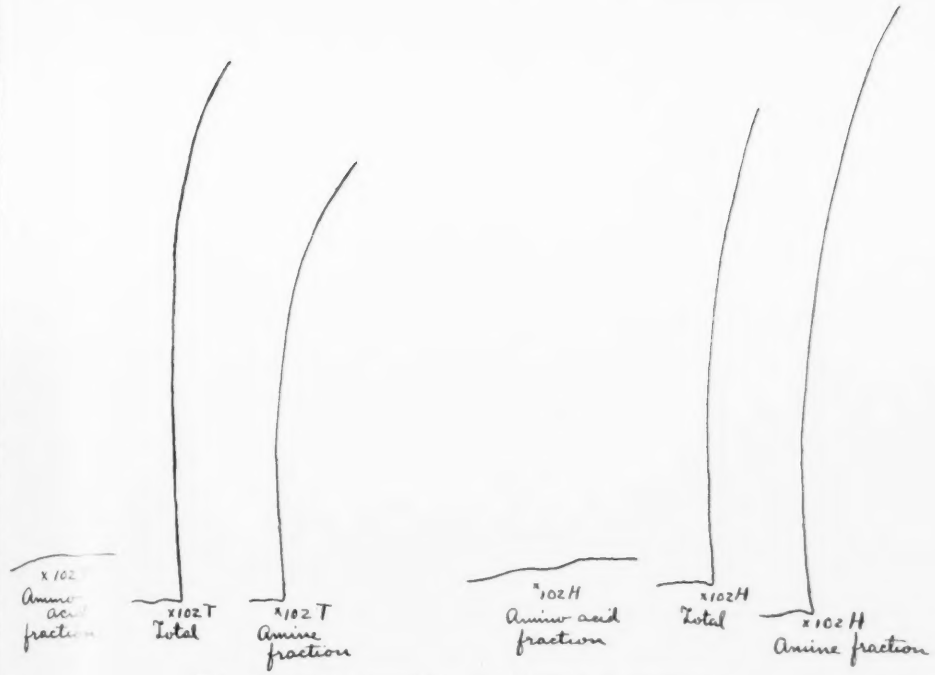


Fig. 12.—Arterial constriction with hemolytic streptococci.

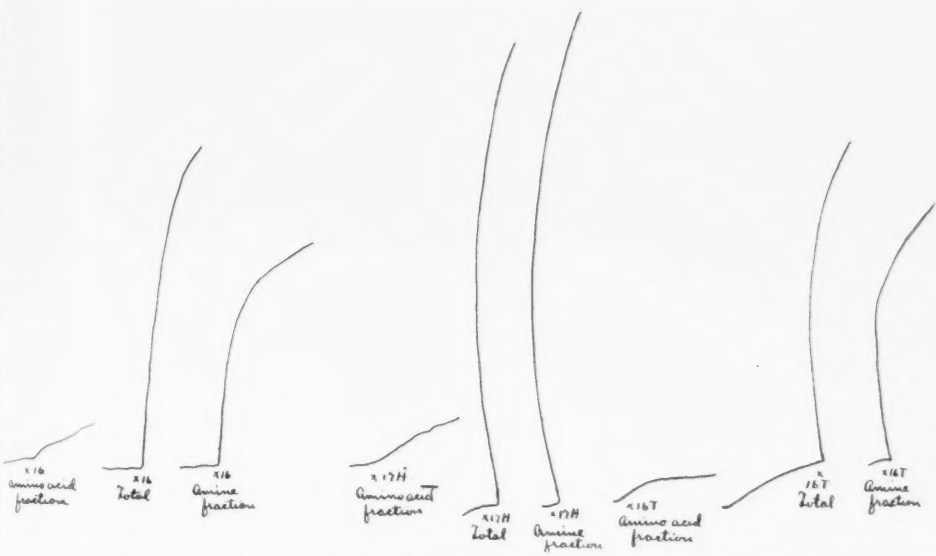


Fig. 13.—Arterial constriction with filtrates from sputums 16 T. J. W. and 17 C. S. (two patients with pneumonia) : histamine was absent.

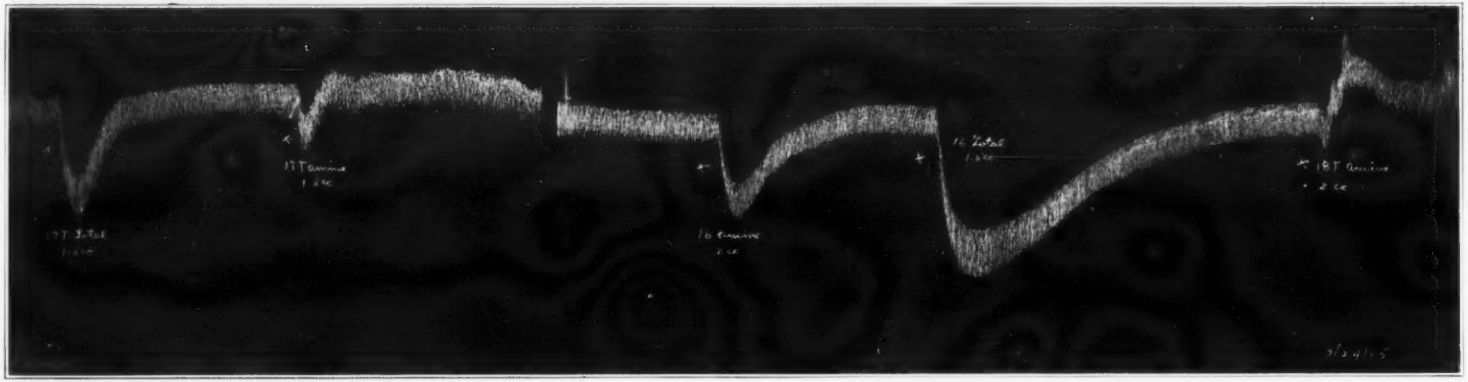
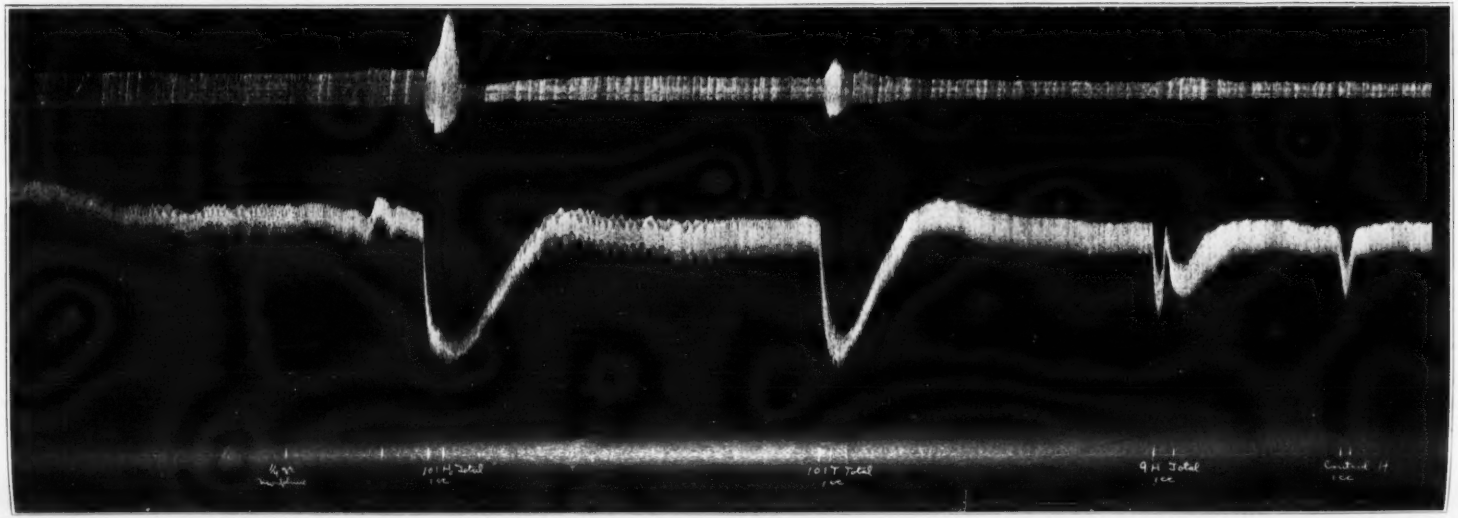


Fig. 14.—Fall in blood pressure obtained with the same fractions that gave arterial constriction in vitro as shown in figure 13.



In our studies on the production of histamine by the colon group, it was found that of seven strains of *Bacillus coli-communior*, three formed histamine; of five strains of *Bacillus coli-communis*, only one formed histamine; of five strains of *Bacillus aerogenes*, one; and of eleven strains of *Bacillus acidi-lactici*, one. No correlation could be found between the faculty of any group to decarboxylate histidine to histamine and any other chemical characteristic, as, for instance, fermentation of various sugars and starches. The same holds true for the formation of pharmacologically active but unidentified substances by *B. coli* in the glycerin-blood broth histidine and tyrosine mediums. The concentrated filtrate of several strains which formed neither histamine nor tyramine contains in some instances a substance that contracts the smooth muscle fibers of the arteries and bronchi, sometimes both, in some cases only one or the other.

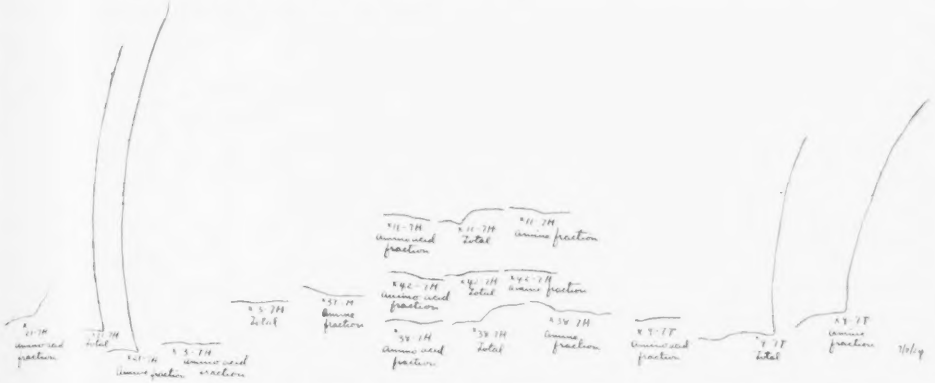


Fig. 16.—Action on arteries in vitro of filtrates from a number of microorganisms isolated from sputum 7 R. A.: Substances produced by organism 9 caused arterial spasm in vitro and a rise in blood pressure in vivo (fig. 17).

Thus a strain of *B. coli-communior* designated as C. S. 2, caused a marked bronchiolar spasm in the pithed guinea-pig and arterial contraction in vitro, while a fraction obtained by the identical chemical procedures from a *B. coli-communior* (designated as S. A. B.) had no physiologic activity on lungs or arteries. Both strains were freshly isolated from human feces.

Another strain of *B. coli* formed a marked bronchospastic poison in the histidine broth, yet not a trace of histamine. The same organism grown in the tyrosine broth did not yield a substance which affected the bronchi.

In the streptococcus group an almost similar behavior is to be observed. Regarding the production of amines of known constitution, we have shown that out of eighteen different strains of streptococci, five

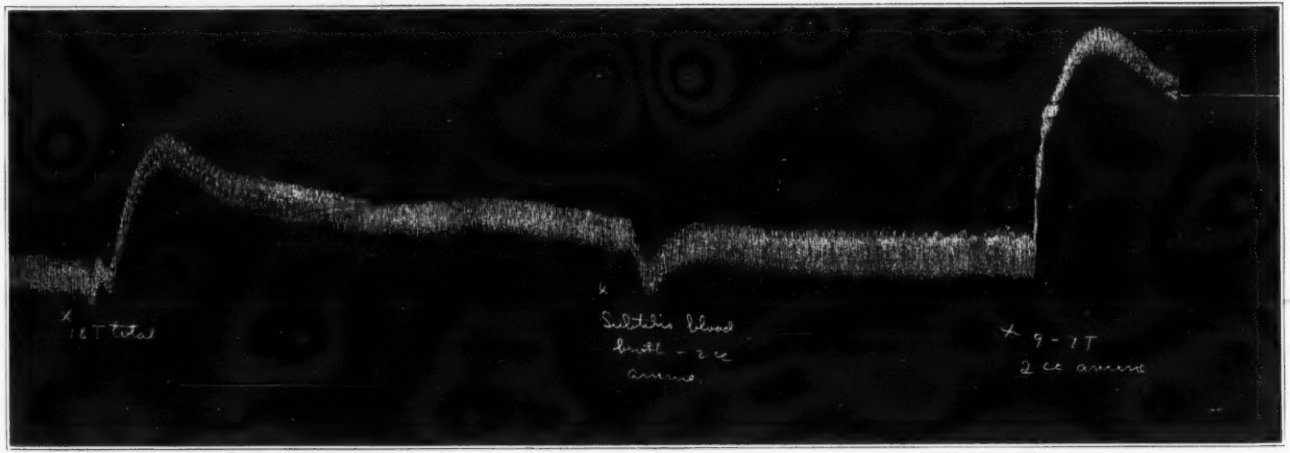


Fig. 17.—Epinephrine-like action of substances produced by organism 9 from sputum 7 R. A.

decarboxylate tyrosine to tyramine. Thirty-five other strains of streptococci were obtained from different diseases by blood cultures, from pus, exudates and focal infections. Of this number, nine yielded a fraction, after cultivation in the broth medium, which was pharmacologically active. Of these nine fractions, four were active on arteries and bronchi, three on arteries alone, and two on bronchi alone (figs. 11 and 12).

It is well known that histamine causes a marked fall in systemic blood pressure; yet in vitro it produces an intense contraction of the arterial strip. The bacterial poisons, chemically not identified and obtained by fractionation of the bacterial filtrates, showed a similar difference between in vivo and in vitro action. Thus, the amine fraction and total filtrate from 17 T (a sputum culture from a patient with lobar pneumonia) showed a marked arterial spasm in vitro (fig. 13); yet the intravenous injection of 2 cc. of the filtrate into the rabbit gave a marked fall of systemic blood pressure (figs. 14 and 15). This bacterial poison behaved, therefore, physiologically like histamine, but chemical examination of the total concentrated filtrate revealed the complete absence of this amine.

On the other hand, another pneumonia filtrate obtained from sputum 18 T produced a marked rise in blood pressure in vivo and a marked arterial constriction in vitro. Its behavior in this respect is therefore comparable to epinephrine, from which it differs, however, pharmacologically by causing intense bronchial constriction. Its reaction recalls tyramine, although chemical tests proved this substance to be entirely absent (figs. 16 and 17).

The toxicity of these unknown poisons for guinea-pigs is marked. On intravenous injection 1 cc. frequently kills a guinea-pig with respiratory failure, while the heart action is often unimpaired for a long time.

By means of continuous dialysis at reduced pressure for twenty-four hours,[2] it is possible to divide the active fractions into a nondialyzable that the active substance is diffusible through a semipermeable membrane residue and the diffusate. The pharmacologic activity resides completely in the latter, while the residue is physiologically without effect, showing and that it is, therefore, a crystalloid

Bacillus typhosus Hopkins when grown in the histidine-glycerin-blood broth medium forms a poison which proved active when tested for bronchiolar and arterial contraction (fig. 18). Histamine was completely absent. This fraction was dialyzed in our apparatus for twenty-four hours. Residue and diffusate were then tested after careful neutralization. Only the latter was physiologically active.

2. Hanke, M. T., and Koessler, K. K.: J. Biol. Chem. **66**:495-499, 1925.

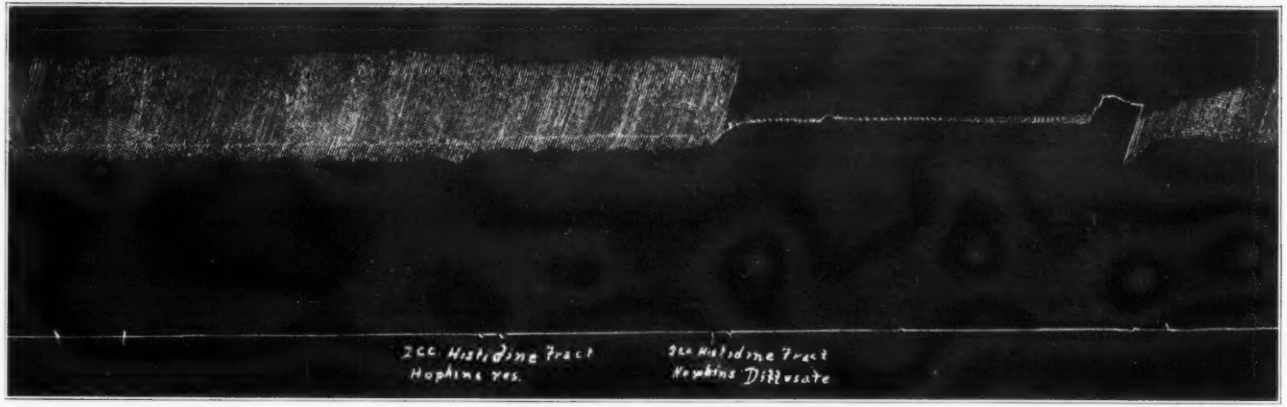

2cc. Histidine Tract
Hopkins Res.
2cc Histidine Tract
Hopkins Diffusate

Fig. 18

Res - Sputum 45
Sputum 45

Fig. 19

The same point is illustrated in figure 19, which was obtained from the culturing of sputum 45 (from a patient suffering from lobar pneumonia) in a tyrosine-blood broth medium. The amine fraction showed intense bronchospasm and arteriolar contraction. Tyramine was completely absent. After dialysis activity could be demonstrated only in the diffusate; the nondialyzable residue was without effect (fig. 20).

Regarding the physical behavior and chemical nature of these poisons involved, no definite statements can be made at this time. They are not destroyed by heating to 100 F., and are not destroyed by treatment with 30 per cent sodium hydroxide solution or by strong acids. Since they pass into amyl alcohol in the presence of alkali they are probably noncarboxylated compounds of the nature of amines.

COMMENT

It could be demonstrated that the mixture of micro-organisms contained in the bronchial secretion (sputum) and in other inflammatory

Fig. 20

Figs. 18, 19 and 20.—Curves showing that the bronchoconstrictor and vasoconstrictor substances produced by bacteria are crystalloids; the nondialyzable residue is pharmacologically inactive.

exudates, e. g., empyema fluid, leads in proper culture mediums to the formation of poisons of definite pharmacologic action. It is evident from our experiments (table 1, experiments 27, 30 and 33) that the formation of these poisons is not dependent on the addition of the amino-acids to our blood-broth-glycerin medium, since they are formed also without their addition. Certain isolated strains of pneumococci, streptococci, *B. coli*, *B. typhosus*, and many other common micro-organisms form such poisons, which have the faculty of contracting blood vessels in vitro and causing bronchospasm in the living guinea-pig. The application of these observations to human pathology might invite the objection that all these poisons formed are the result of autolytic processes of the micro-organisms. That a large number of the organisms are alive in our cultures after fourteen days, we know. Autolytic processes undoubtedly proceed side by side with the life and growth of the micro-organisms. But whatever these soluble poisons are—pure cell

proteins, endotoxins or exotoxins, or products due to autolysis—similar conditions which exist in our in vitro experiments will hold true for their production in vivo. Most foci of infections in the human body, such as tonsils, infected sinuses, sacculated empyemas or the infected bronchial mucosa, may be looked on as tissue cultures in vivo, from which absorption of poisons takes place from time to time. The relation to bacterial poisons of certain forms of dyspnea due to bronchiolar constriction is of great interest to the clinician. The existence of the infectious type of bronchial asthma, or asthmatic bronchitis, whose clinical and immunologic analysis sets it apart from the forms of asthma in which the bronchospasm is produced by an allergic intoxication, finds experimental verification in our observations.

The relation of vascular spasms due to bacterial poisons to certain forms of arterial hypertension receives support by the experiments reported here. Thus, leading clinicians have thought for many years that patients who have had typhoid fever have a tendency to hypertension in later life. Almost all strains of micro-organisms of the typhoid group studied yielded a filtrate that caused intense arteriolar constriction in vitro. But it is the streptococcus, the most ubiquitous of all pathogenic organisms in man, which furnishes the largest number of strains that form vasoconstrictor substances.

SUMMARY

Filtrates from bacterial growth were studied for their pharmacologic action on arteries and bronchi.

The mixture of micro-organisms contained in bronchial secretions (sputum), empyema fluid, tonsils and other foci of infection, when grown in a blood-broth-glycerol-amino-acid medium, forms substances that cause arterial constriction in vitro and bronchial constriction in the living pithed guinea-pig.

Certain strains of many common pathogenic micro-organisms—pneumococci, streptococci, *B. coli, B. typhosus* and *B. paratyphosus*—form substances in a blood broth-glycerin-amino-acid medium which cause arterial constriction in vitro and bronchial constriction in the living guinea-pig.

The action of the poisons is frequently a selective one. A filtrate that causes bronchiolar constriction may have no action on the smooth musculature of the arteries and vice versa. A spasm of both bronchi and arteries is, however, frequently obtained with the same filtrate.

Some poisons causing arterial constriction in vitro cause a marked rise in blood pressure in vivo, thus exhibiting an epinephrine-like behavior. Others which cause constriction of the arteries in vitro lead to a fall of blood pressure in vivo, acting thus like histamine.

The active substances formed are only in a small percentage chemically identifiable as histamine and trymine. In the majority of cases we have to deal with poisons of unknown chemical constitution.

The chemical behavior of the active fraction of the bacterial filtrate suggests that these substances are probably amines.

The bacterial poisons studied may be involved in the production of certain forms of bronchiolar spasm in man (bacterial asthma) and others in the production of certain forms of arterial hypertension.

SPECTROPHOTOMETRIC ANALYSIS OF BLOOD SERUM IN NORMAL AND PATHOLOGIC CONDITIONS

STUDY I *

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As a part of a large group of studies which have been in progress for a number of years at the Mayo Clinic, it has been necessary to learn whether the yellow pigment in the blood serum of patients suffering from pernicious anemia, hemolytic jaundice, obstructive jaundice and severe toxic diseases, such as malaria, was the same substance and whether this pigment was present in smaller amounts in normal blood serum. The answer to this question obviously has much to do with the understanding of the formation of bile and the destruction of erythrocytes.

We have shown that following total removal of the liver,[1] a yellow pigment rapidly accumulates which is found in the blood serum, fat and urine of the animal. Intensive investigation of this phenomenon has led to the conclusion that the substance is bilirubin and is exactly like that found in the serum of animals in which jaundice has been produced by ligation of the common duct.[2] It has been further shown that this pigment is formed chiefly in the bone marrow, spleen and regions rich in reticulo-endothelial cells,[3] and that its formation goes on regardless of the presence of the liver. As a result of this work one may draw the tentative conclusion that erythrocytes are being broken down in the body all the time, chiefly at the site of, or by, reticulo-endothelial cells, and that this hemoglobin is by some process made into bilirubin, which is in turn excreted by the liver as bile. If for any reason the exit of bile is interfered with, it accumulates in the body and produces a condi-

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1. Mann, F. C., and Magath, T. B.: A Further Study of the Effect of Total Removal of the Liver, Am. J. Physiol. **59**:484, 1922; Die Wirkungen der totalen Leberexstirpation, Ergebn. d. Physiol. **23**:212-273, 1924. Mann, F. C.; Bollman, J. L., and Magath, T. B.: Studies on the Physiology of the Liver: IX. The Formation of Bile Pigment After Total Removal of the Liver, Am. J. Physiol. **69**:393-409 (July) 1924.

2. Mann, F. C.; Sheard, Charles; and Bollman, J. L.: Studies on the Physiology of the Liver: XI. The Extrahepatic Formation of Bilirubin, Am. J. Physiol. **74**:49-60 (Sept.) 1925.

3. Mann, F. C.; Sheard, Charles; Bollman, J. L., and Baldes, E. J.: The Site of the Formation of Bilirubin, Am. J. Physiol. **74**:497-510 (Nov.) 1925.

tion of jaundice, being eventually excreted by the kidneys. Thus, one may conclude that if, for any reason, blood is broken down too rapidly to be excreted, jaundice may result, even though there is no dissolution of the continuity of the bile tracts. Indeed, this has been demonstrated by spectrophotometric determinations and the van den Bergh reaction.

In order to test some features of this hypothesis, we have examined a number of serums by spectrophotometric methods. Samples of blood were taken from patients under the most careful conditions to avoid hemolysis. The centrifuge tubes, as well as the syringes and needles, were rinsed out with a physiologic solution of sodium chloride. Blood from the median basilic vein was carefully drawn into the syringe and carefully expelled into the tube. Without agitation, it was allowed to clot and the serum to be expressed in the icebox. The clear serum, after centrifugalizing, was mixed with a mixture of four parts of alcohol and one part of acetone, one part of serum to ten or twenty parts of the alcohol-acetone mixture according to the depth of color of the serum. After standing two hours the mixture was centrifugalized and the clear supernatant fluid analyzed in the spectrophotometer. All cloudy fluids were discarded as well as those showing spectral bands of methemoglobin.

SPECTROPHOTOMETRIC METHODS AND PROCEDURES

The spectrophotometer used in these investigations was one brought out about a year or so ago by Keuffel and Esser and referred to in the literature as a "color analyzer."[4] Figure 1 is a reproduction of a photograph of the instrument. Figure 2 is a cross-section diagram showing the parts of the machine and their arrangement both as to the spectrometric and the photometric operations. The instrument consists essentially of a lamp house carrying two magnesium carbonate blocks (cut from the same cake), which are placed at the rear; these blocks serve as sources of light for transmission through the receptacles containing the liquids to be examined. The beams of light reflected by the blocks, after transmission through two suitable openings in the front of the lamp house, enter the two receptacles containing the solution and the solvent, respectively. These tubes are placed in the proper positions before the entrance slit of the spectrometer by an adjustment on a vertical supporting rod. The spectrophotometer proper does not differ in fundamental principles from the ordinary constant deviation type of instrument except for the addition of a biprism, which is placed in front of the telescopic lens system, and an observing (exit) slit in the eyepiece. Throughout the series of observations which are being reported in this paper the entrance and exit slits were kept at constant or fixed values, following the initial adjustment of the entrance slit to give 100 per cent transmission from the magnesium carbonate blocks throughout the whole of the spectrum. A balance in the intensities of the light transmitted by the standard cell and by the solution under examination was made by means of a sector photometer, the intensity of the light transmitted by the standard cell only being varied. In the majority of the experiments described here, both containing tubes were 10 cm. in length. The standard cell was filled with a mixture of alcohol (four parts) and acetone (one part); the other tube carried the solution to be examined (ordinarily one part of serum to ten or twenty parts of alcohol-acetone solution, depending on the depth of the color).

4. Keuffel, C. W.: A Direct Reading Spectrophotometer, J. Optical Soc. Amer. and Rev. Scientific Instruments **11**:403-410, 1925.

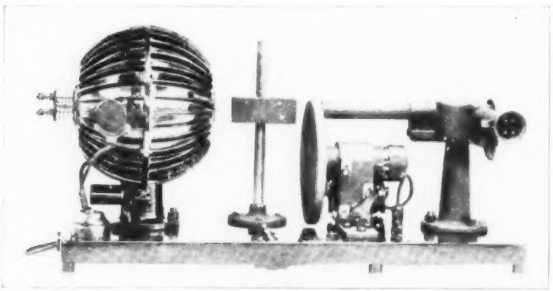

Fig. 1.—Spectrophotometer, or Keuffel and Esser color analyzer.

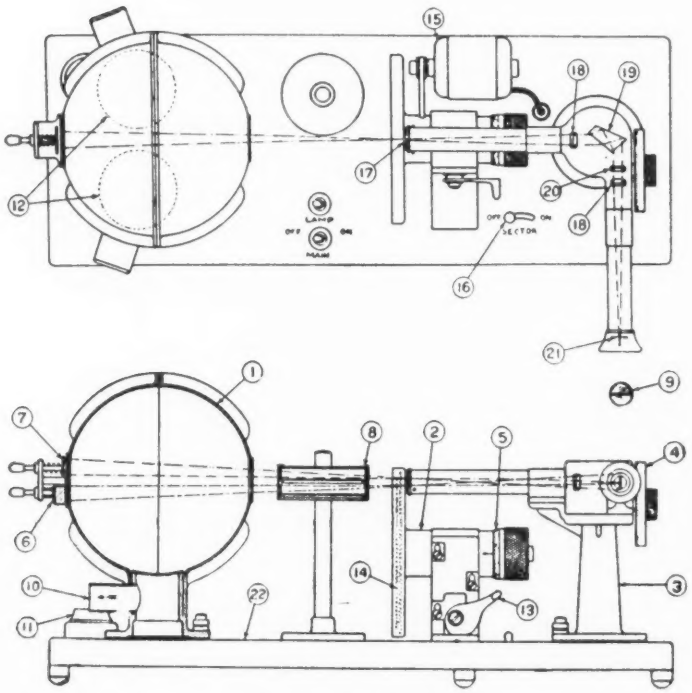

Fig. 2.—Cross-section diagrams of the spectrophotometer: *1*, lamp house; *2*, photometer; *3*, spectrometer; *4*, wave length scale; *5*, photometric scale; *6* and *7*, magnesium carbonate blocks; *8*, field of view through eye slit; *12*, 400 watt lamps; *14*, sector disks; *16*, speed control rheostat; *17*, entrance slit; *18*, collimator objectives; *19*, dispersion prism; *20*, biprism, and *21*, eye slit.

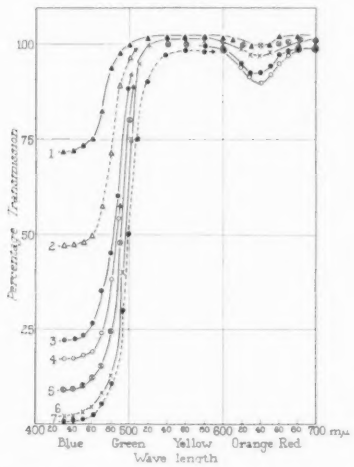

Fig. 3.—Spectrophotometric analyses of blood serums from selected cases, both normal and pathologic: *1*, normal; *2*, normal with higher content of bilirubin; *3*, malaria; *4*, pernicious anemia; *5*, hemolytic icterus; *6*, obstructive jaundice; *7*, bile from a normal person diluted to a degree sufficient to permit of spectrophotometric readings in the region 430 to 460 millimicrons. In the curves *1* to *6*, inclusive, the dilution was kept constant at one part of clear serum to twenty parts of alcohol-acetone mixture.

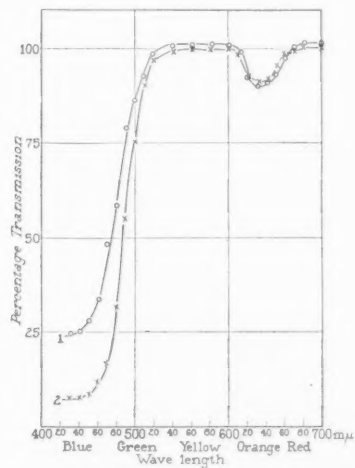

Fig. 4.—Spectrophotometric analysis in a selected case of pernicious anemia: *1*, before transfusion, and *2*, after transfusion. The increased percentages of hematin (620 to 640 millimicrons) and of bilirubin (430 to 500 millimicrons) present in the condition shown in curve *2* as compared with that shown in curve *1* should be noted.

The method of getting the data shown in figures 3, 4 and 5 is as follows: White light is passed through the two containers placed in front of the housing which carries the rotating sectors, and is admitted to the spectrometer. The spectrometer is set at any desired wave length by means of a calibrated wheel. As an illustration, with a setting of 590 millimicrons (which is approximately the wave length of sodium yellow of the spectrum) the observer, on looking through the exit of the eye slit, sees two semicircular colored areas in juxtaposition, with the dividing line horizonal. Both halves of the circle will have the same hue (yellow), but not necessarily the same brightness or saturation value. The adjustment for the equality of brightness, or a match, is then made by varying the size of the sector opening in the rotating sectors placed in front of the spectrometer. The percentage transmission of the solution as compared to the percentage transmission of the liquid (alcohol) in the so-called standard cell is read directly from the calibrated drumhead. This drumhead is mechanically connected to the sectored disks in such a manner as to permit rapid turning of it by hand, thus providing a quick way of varying the relative proportions of open sector and closed sector areas. In determining the percentage

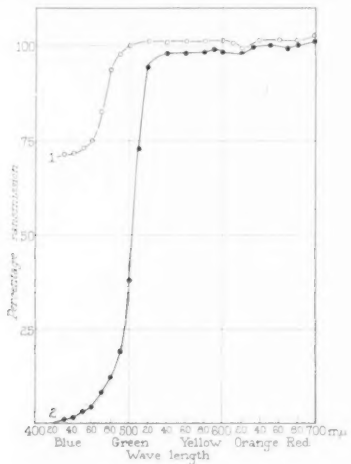

Fig. 5.—Spectrophotometric analyses of blood serums in dilution of one part of clear serum to twenty parts of alcohol-acetone mixture. Curve *1* is for a normal subject; curve *2* gives the results of the analysis in the case of a patient developing an intense jaundice following extensive hemorrhage in the gastrointestinal tract.

transmissions for any given wave length, it has been our custom to allow a few moments for the adjustment of the eye to the spectral hue under observation, and to record as the final reading for each wave length taken (ordinarily by steps of 10 millimicrons) the average of five determinations which do not vary more than from two to three points, respectively. After the measurement for equality of brightness for any given wave length has been made, the procedure, as outlined, is repeated for as many determinations and for as many spectral regions or wave lengths as are deemed necessary. A recent article on spectrophotometric studies on bilirubin[5] gives further details of these methods and the criteria which must be observed if an accurate comparison of data is to be possible.

5. Sheard, Charles; Baldes, E. J.; Mann, F. C., and Bollman, J. L.: Spectrophotometric Determinations of Bilirubin, Am. J. Physiol. **76**:577-585 (May) 1926.

All of the data graphically shown in the curves of figure 3 are drawn on the basis of a uniform dilution of one part of serum to twenty parts of alcohol-acetone mixture in samples of blood from patients covering the range from normal to obstructive jaundice. In the actual experimental work, however, dilutions of from 1:40 to 1:60 were often used for the reason that, in certain instances, such as in cases of obstructive jaundice, a dilution of 1:20 was not sufficiently low to permit of reading the transmissions in the wave length region of 430 to 470 millicrons. It is, however, possible to compute the spectrophotometric transmissions for any specified wave length and for any degree of dilution of solution if one establishes some unit or standard of measurement. For this purpose Bunsen and Roscoe introduced the extinction coefficient, symbolized by ϵ. From Lambert's law $\epsilon = -\frac{\log I}{X}$ in which I represents the intensity of the transmitted light and X the depth of solution. As a standard, therefore, for our spectrophotometric measurements we have taken the value of the transmission I for a constant depth of solution X of 10 cm. and for a given wave length of 440 millimicrons, and for a dilution of 1:20. Wave lengths from 430 to 470 millimicrons can be used; however, the one adopted must be adhered to throughout the investigation. From Beer's law $\frac{\epsilon_1}{\epsilon_2} = \frac{c_1}{c_2}$; hence, the absorption of light by different concentrations of the same substance in the solvent is directly proportional to the concentration C. Therefore, from the laws of Lambert and Beer we are able to say that

$$\frac{\epsilon_1}{\epsilon_2} = \frac{C_1}{C_2} = \frac{-\log I_1}{-\log I_2}$$

It is possible by use of the foregoing formulas to calculate the transmission I_2 for any wave length if C_1, C_2 and I_1 are known. In this manner spectrophotometric data can be reduced to a constant dilution factor of 1:20, as in the curves of figure 3. In practice one can readily determine the spectrophotometric transmission for 440 millimicrons (or other wave lengths), for a specified strength of solution (for example, 1:5 and a depth of 10 cm.) of blood serum from a person chosen as normal. By further dilution to, for example, 1:10, 1:20 and 1:40, and by taking the spectrophotometric readings for the wave length chosen, it is possible to plot on semilogarithmic paper a straight line showing the relationship between the logarithm of the percentage transmission of light ($\log I$) for any specified wave length and the concentration C of the solution.

In general it was found that the amounts of pigment in the serum as shown by this method follow quite closely the amounts demonstrated by the van den Bergh method, except that the latter method does not give differences with accuracy when the amount is small.

RESULTS

As can be seen, the type of the curve in all our cases (figs. 3, 4 and 5), essentially normal persons, from the standpoint of jaundice, and those suffering from pernicious anemia, hemolytic jaundice, obstructive jaundice and malaria, is the same; these curves are exactly like those given by bile and therefore correspond to the spectral analysis of bilirubin. The difference is one of amounts only. If arbitrary units are devised to show the comparative amounts of bilirubin in the serum, one finds that, in the order of amount, the cases are distributed thus: in cases of normal persons, the least amount; in cases of slight anemia and nervous exhaustion, next in amount; then malaria, pernicious

anemia, hemolytic icterus and, finally, obstructive jaundice or intense jaundice without obstruction of the biliary passages. A careful necropsy

The last condition was noted in a patient, and was of particular interest because he presented the apparent paradox of an increasing jaundice without obstruction of the biliary passages. A careful necropsy was performed and particular attention was paid to the liver and its passages, with the result that no specific abnormalities were found. The hemorrhage due to ulcers was of several weeks' duration and bleeding from a large ulcer had been almost continuous, the gastro-intestinal tract being almost completely filled with old blood, and the walls of the intestine markedly discolored. Unfortunately, the record as to whether the reaction to the van den Bergh test was direct or indirect is lacking. Since that time, however, we have seen another case almost exactly like

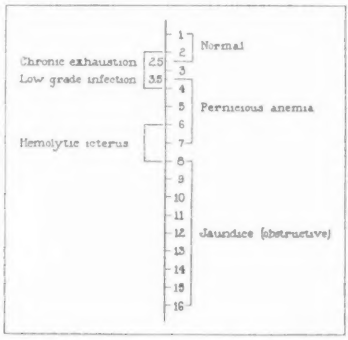

Fig. 6.—Amounts (expressed in arbitrary units) of bilirubin as determined spectrophotometrically in normal and various pathologic conditions.

this one, in which death resulted from gastro-intestinal hemorrhage; the content of bilirubin by the van den Bergh test was even higher, and the reaction was direct. While it is possible that jaundice was due to other factors, necropsy and the clinical study of the cases suggests that jaundice may be due to the absorption of blood through the gastro-intestinal tract. However, numerous experiments have been made which show that hemoglobin is not absorbed through the gastro-intestinal tract, and that the bilirubin content of the blood is not increased by ingestion of whole blood. As is evident in our series of cases, high bilirubin serum content is quite closely correlated with anemia and hemorrhage, and these may be the essential factors in this case rather than absorption of blood through the intestinal wall.

If the low normal content of bilirubin is designated as one unit, then deep jaundice may be represented as from thirteen to seventeen units. Figure 6 illustrates the distribution. It is important to note that there

are no sharp dividing lines and that the values overlap each other. Definite changes in the color of the skin, from a light lemon tint to a deep dark greenish yellow, are seen in all cases having a value of 3.5 units or more.

Almost all curves of serum show a slight absorption, between 610 and 630 millimicrons, which for the present has been interpreted as being due to hematin. Normally this band is faint; in malaria (active

Spectrophotometric Values of Blood Serum

Case	Hemoglobin, per Cent (Dare Method)	Erythrocytes, Millions	Van den Bergh Test, Mg.	Spectrophotometric Values†	Spectrophotometric Value After Transfusion	Van den Bergh Test After Transfusion, Mg.	Hematin	Diagnosis
1	78	4.36	...	1.0	...	....	1—	Migraine
2	40	2.45	...	1.0	...	....	Trace	Myocardial disease, tumor of stomach (?)
3	78	4.50	...	2.0	...	....	Trace	Myocardial disease, constipation
4	75	4.97	...	2.1	...	....	Trace	Congenital club-feet, septic tonsillitis
5	76	4.18	...	2.5	...	....	2	Chronic nervous exhaustion, septic tonsillitis
6	75	....	...	2.6	...	....	1+	Chronic nervous exhaustion
7	65	4.45	...	2.75	...	....	Trace	Chronic tonsillitis, indefinite right-sided pain
8	80	4.58	...	3.0	...	....	1	Chronic nervous exhaustion
9	76	....	...	3.0	...	....	2	Cholecystitis with cholelithiasis; no jaundice
10	77	....	...	3.25	...	....	Slight	Colloid goiter
11	42	3.44	...	3.5	...	....	Slight	Endocarditis; glomerulonephritis
12	30	2.40	1.83*	3.75	4.6	1.36*	3	Pernicious anemia
13	75	4.39	1.5*	4.0	...	....	Slight	Salpingitis
14	52	3.22	2.0*	4.1	...	....	3	Cerebrospinal syphilis; acute malaria
15	30	2.50	...	4.2	...	....	1	Carcinoma of the cecum
16	..	....	2.3*	4.4	...	....	3	Cerebrospinal syphilis; acute malaria
17	25	1.36	....	4.4	4.4	1.5*	3	Pernicious anemia
18	30	2.05	1.81*	4.46	3.56	1.36*	3	Pernicious anemia
19	30	1.93	....	4.5	...	....	3	Pernicious anemia
20	30	1.78	....	4.5	7.6	....	3	Pernicious anemia
21	23	0.98	2.65*	4.75	5.9	2.34	3	Pernicious anemia
22	55	3.13	4.2*	7.0	...	....	1	Hemolytic icterus
23	33	2.00	....	7.5	...	....	3	Pernicious anemia
24	31	2.86	10.4	10.2	...	....	1	Obstructive jaundice
25	65	4.19	14.9	10.72	...	....	2—	Malignant disease of liver with obstructive jaundice
26	54	3.33	15.7	12.5	...	....	1	Obstructive jaundice
27	39	2.74	11.5	16.2	...	....	Slight	Operation for hyperprostatism; severe gastro-intestinal hemorrhage with marked jaundice; septicemia; no biliary obstruction

* Indirect reaction.
† In terms of arbitrary unit.

cases) and pernicious anemia, it is deepest, while in hemolytic and obstructive jaundice it is intermediate. The presence of hematin in serum has been discussed before,[6] and it was pointed out that the more hematin present in blood serum, the more destruction of erythrocytes has taken place.

6. Mann, F. C.; Sheard, Charles; Bollman, J. L., and Baldes, E. J.: The Formation of Bile Pigment from Hemoglobin, Am. J. Physiol. **76**:306-315 (April) 1926.

As can be seen from the accompanying table, there is usually an increase in the bilirubin content of the blood following transfusion in cases of pernicious anemia, which points to the possibility that either some of the red cells are broken down in the patient's body or are injected already hemolyzed as a result of the process of bleeding, mixing with citrate and injection. Since the results of the van den Bergh test are within normal limits in these cases, no help can be expected from the test. It seems worth while studying this further, since some correlation might be established between the amount of hemolysis after transfusion and the degree of reaction on the part of the patient.

There have been many studies comparing the bilirubin of obstructive with that of hemolytic jaundice. One of the most thorough is that of van den Bergh,[7] who came to the conclusion that they were essentially different, basing his conclusions on the Ehrlich diazo reaction. The reaction is direct in obstructive jaundice and indirect in hemolytic jaundice. Also the bilirubin was more readily oxidized in the former case and was more readily adsorbed than the other form to the protein precipitated by the alcohol in performing the indirect test. Grunenberg [8] found a difference in solubility in chloroform between the two types. Hoover and Blankenhorn [9] found that, while "obstructive" bilirubin dialyzed easily, the "hemolytic" bilirubin would not dialyze until alcohol was added. This work was confirmed by Leschke [10] and Brulé, Garban and Weissmann.[11] The nature of the two types of bilirubin has been hypothesized by many. Blankenhorn [12] and van den Bergh both thought that the hemolytic form was linked with a protein or other substance in the serum; Feigl and Querner [13] suggested a lipoid linkage, and perhaps the linkage is with cholesterol as suggested by Rosenthal and Holzer,[14] and by Adler.[15] Adler and Strauss [16] considered the colloid

7. Van den Bergh, A. A. H.: Der Gallenfarbstoff im Blute, Leiden, S. C. Van Doesburgh, 1918.

8. Grunenberg: Aussprache, Verhandl. d. Kong. f. inn. Med. **34**:112-114, 1922.

9. Hoover, C. F., and Blankenhorn, M. A.: Dissociated Jaundice, Arch. Int. Med. **18**:289-303 (Sept.) 1916; also Tr. Am. Phys. **31**:243-260, 1916.

10. Leschke, E.: Aussprache über Ikterus, Berl. klin. Wchnschr. **58**:848, 1921.

11. Brulé, M.; Garban, H., and Weissmann, C.: L'étude de la bilirubine du sérum sanguin; peut-elle aider à reconnaitre la nature d'un ictère, Presse méd. **30**:986-988, 1922.

12. Blankenhorn, M. A.: Acholuric Jaundice, Arch. Int. Med. **27**:131-134 (Jan.) 1921.

13. Feigl, J., and Querner, E.: Bilirubinämie in ihren psychologisch-chemischen Beziehungen mit besonderer Berücksichtigung der diagnostischen Bedeutung, Ztschr. f. d. ges. exper. Med. **9**:153-250, 1919.

14. Rosenthal, F., and Holzer, P.: Beiträge zur Lehre von den mechanischen und dynamischen Ikterusformen: I. Mitteilung, Deutsches Arch. f. klin. Med. **135**:257-280 (March) 1921.

15. Adler, A.: Chemisch-physikalische Untersuchungen an Gallenfarbstoffen und Cholesterin, Verhandl. d. Kong. f. inn. Med. **34**:72-73, 1922.

16. Adler, Erich; and Strauss, Leo: Beitrag zum Mechanismus der Bilirubinreaktion im Blut: II, Mitteilung, Klin. Wchnschr. **1**:2285-2286, 1922.

nature of the two serums as a factor. Andrewes [17] concluded that the two forms were different, basing his conclusion on the van den Bergh reaction, the chloroform solubility test, adsorbability to precipitated proteins, oxidation in iodine, Fouchet's [18] test, dialysis and permeability of the kidneys to it. He thought the difference was not due to linkage to other substances, and that both types were probably present in fresh bile. Either standing or heating caused the obstructive type to go over to the hemolytic type. He offered evidence to support the view that the hemolytic type was identical with hematoidin and that the real difference between obstructive and hemolytic bilirubin lay in a difference in physical state, the latter possibly being in a fine suspension instead of a solution.

Blankenhorn [19] came to the conclusion that bile pigment is frequently found in the blood in cases of pernicious anemia and accounts for the jaundice. He used the tests of Gmelin, Pettenkofer and the spectroscope.

Our experiments show that the spectral analysis of serum obtained from essentially normal persons and of that from persons with jaundice from various causes are alike. Since the serums were treated with an alcohol and acetone mixture before the spectral analyses were made, it might be argued that if there were differences in the bilirubins we had destroyed them in the process of preparation. In other words, it might be contended that we changed all the "direct" bilirubin to "indirect" bilirubin. We tested this by examining specimens in the spectrophotometer before the addition of alcohol, and found the results to be the same as when examined after the addition of alcohol. The blood was allowed to clot and the clear serum was diluted with sodium chloride solution. Of particular interest was the case of hemolytic icterus, which, with an indirect van den Bergh reaction of 4.2 mg. for each 100 cc., gave the same spectrophotometric curve before the addition of alcohol-acetone as after. If the bilirubin contained in blood serums differs, it cannot be shown spectrophotometrically. While it is evident from the van den Bergh reaction that there are some differences in bilirubins, possibly in their physical state or in the nature of a loosely combined state, fundamentally the substance is one and the same whether from so-called obstructive or hemolytic causes, and the common source is evidently the erythrocytes.[20] Furthermore, to argue that the substance

17. Andrewes, C. H.: The Nature of the Difference Between the Bilirubins of Obstructive and Hemolytic Jaundice, Brit. J. Exper. Path. **5**:213-219 (Aug.) 1924.

18. Fouchet, A.: Méthode nouvelle de recherche et de dosage des pigments biliaires dans le sérum sanguin, Compt. rend. Soc. de biol. **80**:826-828, 1917.

19. Blankenhorn, M. A.: The Bile Content of the Blood in Pernicious Anemia, Arch. Int. Med. **19**:344-353 (March) 1917.

20. Mann and Magath (footnote 1). Mann, Sheard, Bollman and Baldes (footnote 6).

in hemolytic icterus is hematoidin is beside the point since Rich and Baustead [21] have shown that hematoidin and bilirubin are identical.

Naturally, it would be important to understand the method by which bilirubin, which must be interpreted as a result of destruction of erythrocytes, is retained in the blood, and certain explanations seem at hand.

A small amount of bilirubin must always be present in the body and represent the daily break-down of erythrocytes in the tissues. This is excreted as bile at a certain rate, which probably varies with the emptying of the gallbladder and the normal activity of the liver. There are probably two methods and a combination of these two that result in retention of bilirubin: the first, too rapid destruction of erythrocytes, and the second, too slow hepatic excretion. Obviously, retention of bilirubin in obstructive jaundice is explained by the mechanical obstruction at the biliary outlet and the damming back of the pigment. Conceivably this may result in increased destruction of erythrocytes, due to the hemolytic action of bilirubin and bile salts, and, indeed, secondary anemia in cases of obstructive jaundice is the rule. Furthermore, it is interesting to note that in obstructive jaundice erythrocytes show an increased resistance, which points to the fact that the more fragile cells have been broken down. This condition also obtains in pernicious anemia.

In hemolytic icterus we deal with an inherited fragile erythrocyte, which probably explains the jaundice, since it is obviously broken down more readily; in many cases there is intermittent obstruction due to small stones. Here, again, the breakdown of erythrocytes results in jaundice which, in turn, increases the breakdown and the vicious circle is established. In malaria rapid mechanical rupture of the erythrocytes, together with probable impairment of the function of the liver, results in the temporary increase of serum bilirubin.

The explanation of the mechanism in pernicious anemia is not at hand, but we offer as a possibility that, due to some cause, perhaps infection, rapid erythrocytic destruction is started in the body, that this resulting increase in the bilirubin content of the blood creates further destruction, and that the vicious circle is thus established. The retention is physiologic as a result of hepatic injury, due to infection and the unusual load it has to carry, and the flood of bilirubin is unable to get out. On this theory one would expect any method of draining the liver or causing a greater output of bile to be beneficial, as has been noted from time to time in the treatment.

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21. Rich, A. R., and Bumstead, J. H.: On the Identity of Hematoidin and Bilirubin, Bull. Johns Hopkins Hosp. **36**:225-232 (April) 1925.

CONCLUSIONS

We have shown that yellow pigment in the blood serum is bilirubin in normal persons, and in those suffering from malaria, pernicious anemia, hemolytic icterus and jaundice due to obstruction of bile. The degree of bilirubinemia varies so that the order named indicates the relative amounts. If the bilirubins are different, it is not shown in the type of spectrophotometric curve.

Usually one hour after transfusion in cases of pernicious anemia, the serum shows an increase in bilirubin.

Any process resulting in too rapid destruction of blood or too slow excretion of bile results in retention of bilirubin. Combinations of both factors probably play a part in the clinical picture and the spectral analysis. Fundamentally all jaundice is hemolytic.

FUNCTIONAL DIASTOLIC MURMURS AND CARDIAC ENLARGEMENT IN SEVERE ANEMIAS*

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The occurrence in patients with pernicious anemia of fatty infiltration of the heart manifested at the necropsy table by the so-called "tigering" of the heart muscle is well known. Dilatation of the heart as well as the presence of so-called "hemic" systolic murmurs are also matters of common observation. These changes are of little clinical interest or importance, so that ordinarily but slight attention is paid to the heart in patients with this disease. The following two cases of pernicious anemia, which presented classical physical signs of chronic valvular heart disease, but in which at necropsy the valves were normal in every respect, illustrate a cardiovascular symptomatology in pernicious anemia which deserves more attention, for it is not unusual.

CASE 1.—G. S., a woman, aged 29, entered Montefiore Hospital, Oct. 5, 1925, with the diagnosis of pernicious anemia. She had been sick for about one year, complaining chiefly of weakness. During this year she had received eight transfusions of blood. On admission she appeared well nourished, slightly dyspneic at rest, with a brownish yellow skin. The hemoglobin was 16 per cent and the red cells numbered 560,000, the white cells 2,400 per cubic millimeter. The differential count showed 82 per cent polymorphonuclears, 16 per cent lymphocytes, and 1 per cent each of eosinophils and basophils. There was macrocytosis, poikilocytosis and anisocytosis. The test meal revealed an achylia gastrica. The heart, both by percussion and by roentgen ray, was slightly enlarged. Noteworthy was the area of dulness in the second interspace just to the left of the sternum, which on the roentgenogram (fig. 1) showed as a prominence of the conus arteriosus. A short presystolic thrill was felt at the apex. The heart sounds at the apex were forcible. The first sound was preceded by a short rumbling presystolic murmur and accompanied by a systolic murmur. The second pulmonic sound was accentuated. In the fourth interspace 1 inch to the left of the sternum a gushing diastolic murmur was audible. There was a systolic murmur at the base. The pulse was full and collapsing; its rate was about 88. The blood pressure was 86 systolic and 42 diastolic. The electrocardiogram showed slight right ventricular predominance. The patient died of bronchopneumonia one week after admission. The necropsy performed by Dr. David Seecof revealed excess iron deposits in the liver and kidneys and fatty infiltration of the heart. The heart weighed 260 Gm., was of average size and had a moderate amount of subpericardial fat. The right side was moderately dilated and strikingly flabby. The tricuspid valve admitted four fingers. There was some dilatation of the pulmonary conus. All the valves were normal. The left ventricle showed no abnormalities aside from "tigering" of the myocardium seen through the thin endocardium. The measurements of the valve orifices were as follows: pulmonary, 7.5 cm.; aortic, 7 cm.; mitral, 8 cm., and tricuspid, 13 cm. The right ventricular wall measured 4 mm.; the left, 10 mm. The coronary arteries were slightly dilated and contained a few scattered yellowish atheromatous plaques.

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CASE 2.—C. A., a woman, aged 33, was admitted to Montefiore Hospital, Aug. 9, 1925, with a diagnosis of pernicious anemia, which had first been made five years before. A splenectomy had been performed two and a half years before her admission to the hospital. She had had eight transfusions of blood. On admission she appeared pale but well nourished, with a brownish pigmentation of the skin. She was orthopneic and had considerable ascites and a massive edema of the legs. The hemoglobin was 12 per cent. The red cells numbered 680,000, the white cells 5,600 per cubic millimeter. There were 48 per cent polymorphonuclears and 46 per cent lymphocytes. There were many nucleated red blood cells. Anisocytosis and macrocytosis were present. Gastric analysis revealed anacidity. The heart was enlarged both to the right and to the left. No thrills were palpable. The first sound at the apex was impure and rumbling and followed by a rough systolic murmur which was transmitted to the axilla. A high pitched inconstant

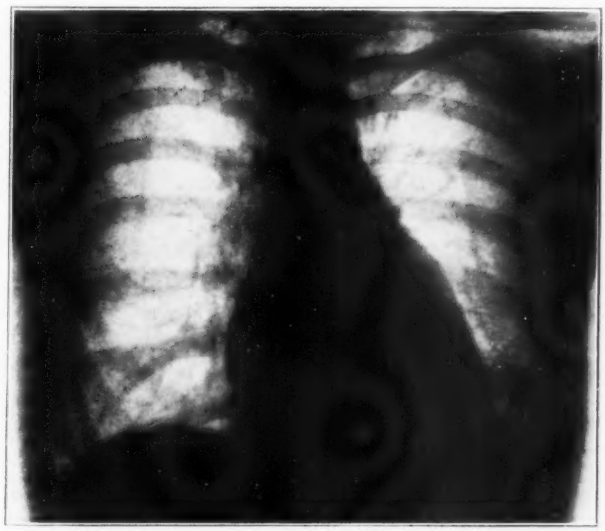

Fig. 1 (case 1).—Heart showing enlargement and prominence of conus pulmonalis.

soft diastolic murmur was audible at the fourth left intercostal space. The cardiac rhythm was regular, the rate 86. The blood pressure was 104 systolic and 44 diastolic. The pulses were full and readily compressible. The teleoroentgenogram (fig. 2) showed a greatly enlarged heart with the following dimensions: median left, 13.4 cm.; median right, 6.7 cm. The electrocardiogram showed left ventricular predominance. The patient continued in the hospital for three and one half months with little change in her condition and finally died from pyelonephritis. Anatomic examination revealed a heart weighing 450 Gm. The epicardial fat was increased in amount. Hypertrophy and dilatation of the left ventricle were marked, of the right ventricle slight. There were no abnormalities of the valves, but the valvular orifices were dilated. The heart muscle was pale but showed no "tigering." Microscopic examination showed slight fatty infiltration of the muscle fibers.

The first of these cases exhibited practically all the classical signs of mitral stenosis, as well as an aortic diastolic murmur, and the second

presented apparently unmistakable signs of aortic insufficiency; yet in both instances all the heart valves were found anatomically intact at necropsy. Although it is common knowledge that systolic murmurs are often of functional origin, diastolic murmurs are usually regarded as rather convincing evidence of organic valvular disease. Isolated cases of diastolic murmurs in the absence of valvular lesions have been described in patients with marked hypertension [1] with dilatation of the aorta; with adherent pleura and pericardium,[1] and with diseases such as actinomycosis of the thorax, which distorts the relationship of the heart and the aorta.[2] The Graham-Steell murmur of relative pulmonic insufficiency belongs in the same category. This murmur is found particularly in patients with severe mitral stenosis,[3] but has been described in various other conditions that raise the pulmonary blood pressure,[4] as well as accompanying an artificial pneumothorax which resulted in a drag on the pulmonary conus.[5]

It appears, however, that functional diastolic murmurs have been noted most often in patients with severe anemias. Friedreich [6] was probably the first to describe a patient with secondary anemia in whom a diastolic murmur was heard during life, and in whom the necropsy revealed normal valves. Subsequently von Noorden [7] described three similar cases, one of which came to necropsy. In one the murmur was diastolic, and in two it was presystolic. In one patient the murmur disappeared as the anemia improved. Sahli [8] observed two patients with severe secondary anemia in whom he found cardiac enlargement as well as a diastolic murmur at the left border of the sternum. He believed that the altered condition of the blood caused the murmur. More recently Cabot [1] and Ortner [2] have reported similar observations in a few patients with pernicious anemia and have suggested that the murmur is determined by a relaxation of the aortic ring. Morse [9] noted

1. Cabot, R. C., and Locke, E. A.: Bull. Johns Hopkins Hosp. **14**:115, 1903. Barié, E.: La Vraie et les Pseudo-Insuffisances Aortiques, Arch. gén. de méd. **1**:257, 1896.

2. Ortner, N.: Ueber akzidentelle diastolische Aortengeräusche, Med. Klin. **19**:408 (March 31) 1923.

3. Steell, G.: M. Chronicle, Manchester, **8**:99, 1888. Internat. Clin. **3**:144, 1898.

4. Litten: Charité Annalen **3**:182, 1878. Becker, E.: Beitrag zur Kenntnis der accidentellen diastolischen Herzgeräusche, Deutsches Arch. f. klin. Med. **121**: 207, 1917.

5. Lublin, A.: Zur Aetiologie akzidenteller diastolischer Herzgeräusche, Deutsch. med. Wchnschr. **47**:1254 (Oct. 20) 1921.

6. Friederich, N.: Virchows Handbuch der specifischen Pathologie und Therapie **2**:227, 1861.

7. Von Noorden, C.: Untersuchungen über schwere Anämien, Charité Annalen **16**:217, 1891.

8. Sahli, H.: Ueber diastolische accidentelle Herzgeräusche, Cor. Bl. f. schweiz. Aerzte **25**:33, 1895.

9. Morse, J. L.: Arch. Pediat. **41**:559 (Aug.) 1924.

diastolic murmurs with water hammer pulse and pistol shot sounds in the arteries in infants and children with marked anemia, and observed their disappearance as the condition of the child improved. Finally, Kraus [10] heard diastolic murmurs in eight of forty-seven patients with pernicious anemia which he personally observed, and found them reported in ten of eighty-three cases that he collected from the literature.

We have reviewed the case histories of thirty-nine patients with pernicious anemia who have been treated at Montefiore Hospital. Diastolic murmurs were noted in six cases of this series. The murmur usually was heard best at the fourth intercostal space to the left of the sternum, but sometimes was apical. It was inconstant, but its disappearance was not always paralleled by a change in the blood picture or in the condition of the patient. One other patient presented an apical rumbling presystolic murmur, and two of the patients with aortic diastolic murmurs had apical presystolic murmurs as well.

Enlargement of the heart in anemic patients has often been noted. In the earlier literature there are many references to increased heart size in chlorosis. For instance, Gautier [11] found cardiac enlargement clinically in twenty of twenty-two patients with severe chlorosis. He noted, too, that with the increase in red blood cells and hemoglobin the heart rapidly receded in size. Subsequent studies revealed cardiac enlargement in many patients with pernicious anemia. Kraus [10] found cardiac dilatation in thirty of his forty-seven cases of pernicious anemia. The heart was enlarged to percussion. Large hearts, unexplained by valvular lesions or hypertension, were found by Wallgren [12] in six of seven, and by Cabot [13] in twenty-two of twenty-three patients with pernicious anemia who came to necropsy, and Strieck [14] reported that 60 per cent of a series of 165 patients with pernicious anemia had enlarged hearts. Of the thirty-nine patients observed at Montefiore Hospital, twenty-three had enlargement of the heart to the left, occasionally to the right, as determined by percussion. In six instances in which teleoroentgenograms were taken, this increase in heart size was verified objectively. All the twelve cases that came to necropsy showed cardiac dilatation. The average heart weight was 330 Gm., the smallest

10. Kraus, F.: Die klinische Bedeutung der fettigen Degeneration des Herzmuskels schwer anämischer Individuen, Berl. klin. Wchnschr. **42**:V, 1905.

11. Gautier, E.: Ueber die morphologischen Veränderungen des Herzens bei der Chlorose auf Grund klinischer Beobachtungen, Deutsches Arch. f. Klin. Med. **62**:120, 1899.

12. Wallgren, A.: Die Arterien der Niere und der Blutdruck, Acta med. Scandinav. **56**:356, 1922.

13. Cabot, R. C.: Facts on the Heart, Philadelphia, W. B Saunders Company, 1926, p. 19.

14. Strieck, F.: Zur Symptomatologie der Biermerschen Krankheit., Med. Klin. **20**:1538 (Nov. 2) 1924.

250, the greatest 460 Gm. Only three hearts weighed less than 300 Gm. Yet there was little apparent hypertrophy of the ventricular walls. The dilatation involved all the chambers of the heart, including the pulmonary conus in four cases. "Tigering" of the myocardium was present in only one-half the cases. In all instances the valves were normal, except for small atheromatous flecks on the aortic leaflet of the mitral valve in a few cases.

The mitral configuration of the heart with bulging or prominence of the so-called left auricular salient in the roentgenogram, which was

Fig. 2 (case 2).—Heart showing great enlargement and mitral configuration.

noted by Kraus in 10 per cent of his patients with pernicious anemia, is well shown in our illustrations.

That heart changes similar to those that have been described may accompany severe secondary anemia as well is evidenced by the following two cases:

Case 3.—A. N., a man, aged 53, was admitted to Montefiore Hospital, Nov. 18, 1918, complaining of epistaxis, dyspnea, precordial oppression, vertigo and polyuria. These symptoms dated from an attack of pneumonia one year previously, although he had had frequent copious nosebleeds for about ten years. The patient was pale and thin. Scattered over the skin, the tongue and the nasal mucosa were many small telangiectases from 1 to 6 mm. in diameter. The lungs showed emphysema and bronchitis. The heart was not much enlarged to percussion but roentgen-ray

examination revealed enlargement to the left but no aortic widening. Systolic murmurs were heard at the apex and at the base. The liver was large and nodular. The pulse rate was between 80 and 90; the blood pressure was 118 systolic and 70 diastolic. The blood Wassermann reaction was negative. The coagulation time of the blood was twelve minutes. The blood examination on admission revealed 40 per cent hemoglobin and 2,500,000 red cells. Two years later, shortly before death, the hemoglobin percentage was 20 and the red blood cells numbered 790,000. The white cells numbered 4,800 with 59 per cent polymorphonuclears and 41 per cent mononuclears. There was moderate poikilocytosis, but there was no macrocytes. The patient continued to have frequent severe nosebleeds in spite of all therapy and finally succumbed, Feb. 11, 1921, to the effect of a severe anemia. Necropsy revealed numerous gummas of the liver with syphilitic cirrhosis. The heart was greatly enlarged, weighing 680 Gm. The right auricle and ventricle

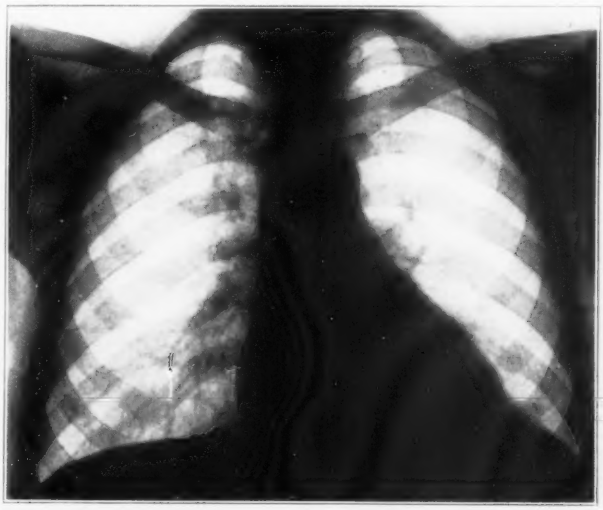

Fig. 3 (case 4).—Heart showing enlargement and mitral configuration.

were strikingly dilated, while the left auricle and ventricle showed moderate dilatation. The right ventricle measured 4 mm., the left 12 mm. in thickness. The valves were normal. There was considerable "tigering" of the myocardium. The coronary arteries showed scattered atherosclerotic plaques. The kidneys together weighed 280 Gm. Their surfaces were smooth and their cortices wide. The lungs showed moderate emphysema.

Case 4.—E. H., a man, aged 40, was admitted to Montefiore Hospital, March 17, 1925, complaining of weakness and loss of weight of one year's duration. Physical examination revealed a mass on the inner aspect of the right ilium, marked yellowish pallor, and signs of funicular myelitis. The hemoglobin was 18 per cent, the red cells numbered 960,000. There were no nucleated forms or macrocytes. There were 11,000 white blood cells, with 83 per cent polymorphonuclears, 12 per cent lymphocytes, 2 per cent mononuclears and 3 per cent myelocytes. The heart was slightly enlarged to the left with an apical systolic murmur. The blood pressure was 100 systolic and 70 diastolic. The patient died, Aug. 7, 1925. Necropsy revealed a large retroperitoneal sarcoma. The heart weighed 400 Gm.

There was much epicardial fat. The right auricle and ventricle were dilated. The right ventricle measured 5 mm. and the left 10 mm. in thickness. The valves were normal.

Dilatation of the heart in severe anemia is due most probably to the deficient oxygen supply to the cardiac muscle. In another connection Lewis [15] has suggested that an inadequate blood supply to the heart will cause it to dilate. Lüdke and Schüller [16] were able to demonstrate cardiac dilatation in dogs that were subjected to experimental anemia. The diastolic murmur that is heard apparently only in patients with far advanced anemia whose hearts are enlarged is caused most probably by the accompanying stretching and relaxation of the aortic ring. The origin of a presystolic murmur is less readily understood. The observations of Fahr and Ronzone,[17] while not absolutely conclusive, suggest a greatly increased minute volume flow as well as an accelerated blood velocity as a compensatory phenomenon to the lessened oxygen carrying power of the blood in severe anemias. It is readily conceivable that this might increase the work of the heart to a degree sufficient to cause hypertrophy of the ventricular chambers. The relative frequency of marked right ventricular dilatation in our series of cases is worthy of note but not readily explained.

SUMMARY

Cardiac dilatation, often accompanied by cardiac hypertrophy, is a common finding in patients with severe anemias. Diastolic murmurs resembling those of aortic insufficiency are heard in about 10 per cent of patients with pernicious anemia. These are caused not by organic valvular disease but by a relative insufficiency of the aortic valve. In a smaller number of anemic patients presystolic murmurs may be present in the absence of mitral stenosis. In patients with severe anemia the presence of cardiac enlargement, a mitral configuration of the heart, and a diastolic or presystolic murmur does not warrant the diagnosis of organic valvular disease.

15. Lewis, T., and Drury, A. N.: Observations Relating to Arteriovenous Aneurysm, Heart **10**:301, 1923.

16. Lüdke, H., and Schüller, L.: Ueber die Wirkung experimenteller Anämien auf die Herzgrösse, Deutsches Arch. f. klin. Med. **101**:512, 1910.

17. Fahr, G. E., and Ronzone, E.: Circulatory Compensation for Deficient Oxygen Carrying Capacity of the Blood in Severe Anemias, Arch. Int. Med. **29**:331 (March) 1922.

SICKLE CELL ANEMIA

REPORT OF A CASE GREATLY IMPROVED BY SPLENECTOMY, EXPERIMENTAL STUDY OF SICKLE CELL FORMATION *

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The term "sickle cell anemia" is used to designate two conditions: (1) a hereditary predisposition to anemia occurring only in the negro race and characterized by the development of a crescentic distortion of the red corpuscles under certain conditions, and (2) a chronic, severe anemia affecting only negroes with this predisposition, characterized by remissions and exacerbations, hemolytic jaundice, episodes of abdominal pain, joint pains without inflammation and a tendency to ulcer of the leg.

In 1910 Herrick [1] reported a case of a negro who had jaundice, a history of ulcer of the leg, leukocytosis and severe anemia associated with the occurrence of crescentic red corpuscles in the stained and wet smears of the blood. Some of the crescentic poikilocytes were nucleated.

Washburn [2] reported the second case with essentially the same clinical picture, and Cook and Myer,[3] the third. Cook and Myer believed that the condition constitutes a clinical entity, and that its incidence is familial.

Emmel [4] published separately a study of the third case, reporting that the number of sickle cells in sealed wet smears of the patient's blood increased on standing; that during remissions when stained smears showed no sickles, such forms appeared in sealed wet smears; and that sickles developed in such preparations of the blood of the patient's

* From the Laboratory of Surgical Pathology, Indiana University School of Medicine.

1. Herrick, J. B.: Peculiar Elongated and Sickle-Shaped Red Blood Corpuscles in a Case of Severe Anemia, Arch. Int. Med. **6**:517 (Nov.) 1910.

2. Washburn, R. E.: Peculiar Elongated and Sickle-Shaped Red Blood Corpuscles in a Case of Severe Anemia, Virginia M. Semi-Month. **15**:490 (Feb.) 1911.

3. Cook, J. E., and Myer, J.: Severe Anemia with Remarkable Elongated and Sickle-Shaped Red Blood Cells and Chronic Leg Ulcers, Arch. Int. Med. **16**:644 (Oct.) 1915.

4. Emmel, V. E.: A Study of Erythrocytes in a Case of Severe Anemia with Elongated and Sickle-Shaped Red Blood Corpuscles, Arch. Int. Med. **20**: 586 (Oct.) 1917.

clinically healthy father. Emmel noted evidence of phagocytosis of the corpuscles by large mononuclear cells in the stained smears. He found no nucleated sickle cells, and concluded that sickle cells are formed in the circulating blood. He believed that the sickle distortion, which he regarded as specific for this type of anemia, was the result of an accentuation of the factors normally involved in the transformation of the original spherical erythrocytes into biconcave disks.

Mason,[5] reporting the fourth case, first used the term "sickle cell anemia."

Sydenstricker, Mulherin and Houseal[6] added cases five and six, the latter with an autopsy report. The nonfatal case was the first in which the spleen was enlarged. The occurrence of nucleated sickle cells in the peripheral blood was again affirmed. Attacks of abdominal pain were stressed as a prominent symptom and related to splenic pathologic change. Sickle cells were found in the tissue sections of the case which came to autopsy, and were particularly numerous in smears of the bone marrow. The manner of fixation was not stated explicitly, but liquor formaldehydi was mentioned with reference to one tissue. The authors concluded that sickle cells are preformed in the bone marrow, and that the anemia is hemolytic.

Huck,[7] reporting cases seven and eight, stated for the first time that sickle cells in wet smear cultures revert to the circular form after a variable period of time. Washed corpuscles suspended in physiologic sodium chloride and citrate solution became sickle cells as readily as in the presence of serum. Phagocytosis of red cells by large mononuclears was observed in stained smears. Huck submitted brief notes of symptoms and blood observations on twelve other persons, eleven of whom belonged to one family. Sickle cells were found in stained smears of the blood of those with impaired health and anemia. In those with slight or no symptoms and practically normal red cell counts, the sickle cell tendency was proved by observation of wet smear cultures. The completeness of the sickle cell formation was roughly proportional to the severity of the symptoms. Huck, on this evidence, introduced the concept of latent sickle cell anemia. A study of the families convinced him that the disease is inherited as a dominant mendelian trait.

In 1924 Sydenstricker[8] summarized observations on his series of eighty cases distributed among ten families. In the entire series seventy-

5. Mason, V. R.: Sickle Cell Anemia, J. A. M. A. **79**:1318 (Oct. 14) 1922.

6. Sydenstricker, V. P.; Mulherin, W. A., and Houseal, R. W.: Sickle Cell Anemia; Report of Two Cases in Children with Necropsy in One Case, Am. J. Dis. Child. **26**:132 (Aug.) 1923.

7. Huck, J. G.: Sickle Cell Anemia, Bull. Johns Hopkins Hosp. **34**:335 (Oct.) 1923.

8. Sydenstricker, V. P.: Further Observations on Sickle Cell Anemia, J. A. M. A. **83**:12 (July 5) 1924.

one patients had latent sickle cell anemia, and eleven of these had a palpable spleen and five had ulcer of the leg. In the patients with active anemia, the liver was regularly enlarged, the spleen was palpable only when there was a history of malaria, and the legs showed ulcers, or scars of ulcers, and edema. Red cell resistance was increased, bilirubinemia was marked, and urobilin was increased in the urine and stools. The urine did not contain bile. Phagocytosis was observed in stained blood smears of patients with active and latent anemia and wet smear cultures showed sickle cell formation with phagocytosis of the sickle cells. The statistical evidence of hereditary transmission was reinforced by finding sickle cells in the blood from the umbilical cord in two patients. The phenomenon of auto-agglutination was observed in four patients. The summary of five autopsies included the following points: enlarged liver with pigment and degeneration; atrophic spleen with decrease of pulp and follicles, increase of trabecular tissue, fine pigment, deposits of iron containing pigment, recent and old infarcts and overfilling with blood irregularly distributed; hyperplastic bone marrow, and the lesions of intercurrent infections. Sydenstricker concluded that the disease is probably a hereditary defect in the spleen and blood-forming tissues resulting in an abnormality of the red cells predisposing them to hemolysis and phagocytosis, and that when anemia is present it is caused by an activation of hemolysis by factors innocuous to normal persons.

Graham,[9] reporting the ninth case, stressed the fact that sickle cell formation in wet smear cultures is inconstant. Slides prepared in the same way and at the same time yielded both positive and negative results. The case came to autopsy and Graham was able to report that the red corpuscles of the tissues fixed in liquor formaldehydi were all sickle cells, but that those of the tissues fixed in Zenker's solution were normal in shape. Graham regarded the latent phase of the disease as a "status hemicus." The excitant provoking the active phase he considered to be probably a metabolic disturbance or an infection.

Moser and Shaw[10] reported the tenth case, the first in a "northern" negro. The patient had never been out of Nebraska, and his family had lived in the North for several generations. The authors found that the blood serum of their patient did not hemolyze normal red cells.

The subject of Anderson's report,[11] the eleventh case, was a Pennsylvania negro. Anderson confirmed Graham's experience of the capriciousness of sickle cell formation in wet smear cultures.

9. Graham, G. S.: A Case of Sickle Cell Anemia with Necropsy, Arch. Int. Med. **34**:778 (Dec.) 1924.

10. Moser, A. R., and Shaw, W. J.: Sickle Cell Anemia in a Northern Negro, J. A. M. A. **84**:507 (Feb. 14) 1925.

11. Anderson, H. B.: Sickle Cell Anemia, Report of an Active Case, Am. J. M. Sc. **171**:641 (May) 1926.

Hamilton [12] reported the twelfth case which was complicated by gallstones, for which laparotomy had been performed, and Browne [13] reported the thirteenth.

The case presented here is the fourteenth to be reported in detail, the first to be reported from Indiana, and the first to be reported in which the patient was treated by splenectomy.

REPORT OF CASE

History.—Curtis C., colored, aged 4, was brought to the James Whitcomb Riley Hospital, Dec. 23, 1925. His mother said that for months he had been tired and sleepy. During this time he had had frequent attacks of epistaxis and had complained of headache; he had also had intermittent attacks of epigastric pain associated with vomiting. His health had never been good. He had had bronchopneumonia at the age of 8 months, rheumatism with swelling of the elbow joints some months later, pertussis at the age of 1 year, and both measles and influenza at the age of 3. The mother was not in good health. The father and three sisters were in good health. The maternal grandfather died of heart trouble. The maternal grandmother was living but afflicted with tuberculosis. The paternal grandparents were in good health. There was no history of jaundice or ulcer of the leg.

Examination.—The patient was fairly well nourished. The sclera showed a greenish color. The mucous membranes were pale, the teeth carious, the tonsils small but inflamed. The lymph glands of the neck were moderately enlarged; of the axillary and inguinal regions, slightly enlarged. On the left side, lung resonance was impaired, moist râles were heard, and a friction rub was present in the axillary line at interspaces 6, 7 and 8. The right lung was apparently normal. The maximal impulse was in the nipple line at interspace 5; the apex beat, 2 cm. lateral to the nipple line. A loud blowing systolic murmur, heard best at the apex, was transmitted to the axilla. The second sound was loud and snappy. The abdomen was distended and tympanitic on the right; on the left, it was occupied by the spleen which extended to the crest of the ilium. The spleen was firm and not tender. Its notch was distinctly felt. The liver was palpable 2 fingerbreadths below the costal border. The rectal temperature varied daily between 98.6 and 100.5 F.; the pulse rate between 90 and 120. A febrile crisis, reaching a peak of 104.5 F. occupied a period of five days and was unattended by signs suggestive of its cause. (Laboratory data susceptible of tabulation are given in tables 1 and 2.) The urine was dark but normal to the ordinary tests, including the shake test for bile. The stool was normal except for its high content of urobilin; there were no ova, parasites or undigested food. The blood Wassermann reaction was negative twice; blood culture was negative, and autoagglutination was negative. The whole blood had a cholesterol content of 81 mg. per hundred cubic centimeters, as estimated by the method of Leiboff. The coagulation time was seven minutes and the bleeding time was two minutes. Wet smears of the fresh blood contained a few more or less crescentic poikilocytes. Typical sickle cells developed in sealed wet smears in twenty-four hours at room temperature and reverted to a circular form in two weeks.

Operation and Result.—The patient's symptoms were all explicable on the ground of severe anemia, except for the attacks of epigastric pain which we attributed to pathologic change in the spleen. The laboratory data confirmed the clinical diagnosis of severe anemia and established the presence of acholuric

12. Hamilton, J. F.: A Case of Sickle Cell Anemia, U. S. Veterans' Bureau M. Bull. **2**:497 (May) 1926.

13. Browne, Earl Z.: Sickle Cell Anemia, M. Clin. N. Amer. **9**:1191 (Jan.) 1926.

jaundice of hemolytic origin. The tremendous rate of blood destruction was revealed by the enormous output of urobilinogen and urobilin (estimated together). The febrile course and leukocytosis were held to establish the presence of an infection which might be either etiologic or intercurrent in its significance. The high leukocyte counts, the high percentage of reticulocytes and the presence in the circulating blood of nucleated red cells were taken to indicate hyperfunction of the hematopoietic system, presumably compensatory. Thus far, the case was considered typical of acquired hemolytic jaundice. However, one datum, the increased resistance of the red cells, was inconsistent with that diagnosis. Finally, the demonstration of sickle cells was held to establish the diagnosis of active sickle cell anemia.[14]

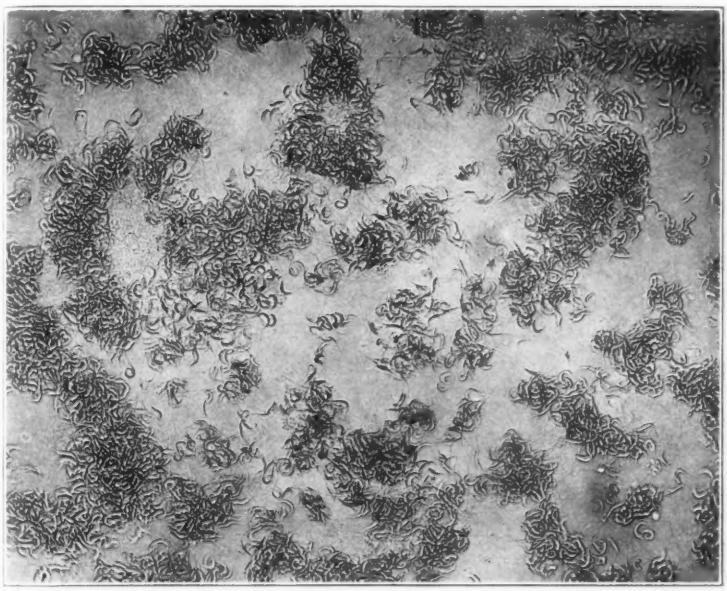

Fig. 1.—A wet smear culture of the patient's blood forty-eight hours after preparation. Practically all of the red corpuscles are sickle shaped. The apparent colonization of the corpuscles around granular areas has not been reported previously.

Sydenstricker[15] and Huck had suggested that splenectomy might be beneficial in sickle cell anemia. We considered it especially indicated in this case because of the conditions stated in the foregoing and because the patient's red cell count was steadily falling. An effort to find a compatible donor for a preliminary blood transfusion was unsuccessful, and the patient's sole therapy was splenectomy.[16]

14. The assistance of Dr. J. O. Ritchey and of Dr. J. Don Miller, Department of Medicine, Indiana University School of Medicine, is acknowledged.

15. Sydenstricker, V. P.: Sickle Cell Anemia, South. M. J. **17**:177 (March) 1924. The opinion of J. G. Huck was given in his discussion of this paper.

16. The operation was performed by Dr. Murray N. Hadley, Department of Surgery, Indiana University School of Medicine.

Immediate convalescence was uneventful. Clinical improvement later was rapid. The tables show that the red cell count rapidly increased and that the output of urobilinogen-urobilin rapidly decreased. For one month after operation it was impossible to detect sickle cells in sealed wet smears prepared exactly as before operation. Later they could be found as regularly as before operation. One fragility test after operation showed a decrease of resistance. A subsequent test again showed increased resistance which, however, was at a level nearer the normal than the preoperative values. The patient at the present time is symptomatically well and shows no sign of icterus. However, he still has the sickle cell trait.

Pathologic Changes in the Spleen.—After removal and in the fresh condition the spleen was about three times the normal adult size. After twenty-four hours of fixation in formaldehyde, the organ measured 14 cm. from pole to pole.

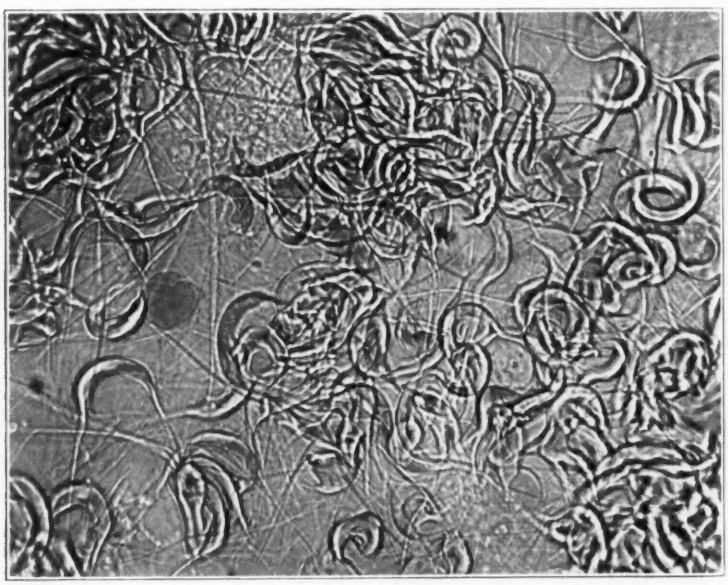

Fig. 2.—An oil immersion view of the specimen represented in figure 1. Under working conditions, practically all the filaments could be traced to the horns of sickle shaped corpuscles.

In the fresh condition the spleen was soft and a dark purplish color. The surface was coarsely pebbled, and the margins were more obtusely rounded than normally, giving the impression of turgidity. The vessels of the hilum presented no calcifications, thrombi or abnormal thickenings.

The spleen cut with less than normal resistance. The capsule was about twice the normal thickness, and there were opaque patches of still greater thickness. The parenchyma was extremely soft and of a dark red color. Malpighian bodies were not visible.

Under aseptic conditions, a sample of the pulp was taken for bacteriologic culture, and two blocks were cut for histologic examination. One of the blocks was fixed in 4 per cent formaldehyde and the other in Zenker's solution. Both were dropped into the fixing solutions within ten minutes of the delivery of the organ from the abdomen.

The tissue culture yielded a green-producing streptococcus in pure culture.

The following microscopic observations were made on the tissue fixed in formaldehyde except where it is explicitly stated otherwise. Hematoxylin and eosin preparations showed a slight thickening of the capsule but no increase in trabecular tissue. On the contrary, the trabeculae were spaced farther apart than in the normal organ. Lymphoid follicles were almost totally lacking, and there was a general paucity of lymphocytes. The tissue was overstuffed

TABLE 1.—*Blood Cell Counts, Hemoglobin Determinations, Differential Formulas and Fragility Tests Before and After Splenectomy*

Date	Red Blood Cells	Hemoglobin	Color Index	White Blood Cells	Lymphocytes	Large Mononuclear Transitionals	Neutrophilic Polymorphonuclears	Eosinophilic Polymorphonuclears	Basophilic Polymorphonuclears	Nucleated Red Cells per 100 White Blood Cells	Reticulocytes, per Cent	Platelets	Limits of Hemolysis Sodium Chloride, per Cent	
													Patient	Control
2/24	2,110,000	30	0.72	13,000	40	5.5	45.5	0.5	1.5	7	..	.....	.....	.....
2/30	2,000,000	30	0.75	12,000	42	7.0	49	1.0	1.0	7	..	.....	0.32-0.20	0.48-0.40
3/ 4	1,850,000	..	....	18,000	40	5.0	54	1.0	0	11	19	.....	0.30-0.22	.....
3/10	1,936,000	..	....	.....	..	...	....	...	...	..	..	253,000	.....	.....
3/15	.....	..	....	.....	..	...	....	...	...	..	..	.....	0.30-0.20	0.50-0.40
3/19	Splenectomy													
3/20	.....	..	....	28,000	..	...	....	...	...	..	..	.....	.....	.....
3/24	2,256,000	..	....	16,400	..	...	....	...	...	..	..	160,000	.....	.....
3/ 8	4,072,000	35	0.48	17,000	..	...	....	...	...	..	..	224,000	0.60-0.50	.....
3/21	4,432,000	40	0.45	25,000	..	...	....	...	...	..	..	.....	0.40-0.30	.....

TABLE 2.—*Bilirubin in the Blood Serum and Urobilin in the Excreta Before and After Splenectomy* *

Date	Serum Bilirubin (Van den Bergh Reaction)				Urobilin Excretion†	
	Direct		Indirect		Urine, Mg. per 24 Hours	Stool, Mg. per 24 Hours
	1 Minute	1 Hour	Mg. per Liter	Units		
1/ 1	—	+	13.4	2.7	...	.....
1/ 8	—	+	14.5	2.9	...	.....
1/17	..	..	....	...	40#	.....
1/18	..	..	....	...	157	18,200§
1/19	..	..	....	...	101	.....
1/19	Splenectomy					
1/27	..	..	....	...	...	623
2/ 2	..	..	....	...	58	607
2/ 3	..	..	....	...	30	22
2/ 7	..	..	....	...	18	379
2/ 8	—	±	1.8	0.3	41	245
2/ 9	..	..	....	...	38	.....
2/10	..	..	....	...	...	205

* All chemical analyses were made by Dr. R. N. Harger, Department of Biochemistry, Indiana University School of Medicine.

†In the method of Ehrlich and McMasters: Urobilin Physiology and Pathology: Quantitative Determination of Urobinin, J. Exper. Med. **41**: 503 (April) 1925, which was used, the urobilinogen is converted into urobilin, and both are therefore estimated with each other. A nephelometer was used in place of a color comparator box. The acriflavine was purchased from Coleman and Bell.

‡ This value was obtained on a stale specimen.

§ This value was obtained for a bulky specimen which is thought to have represented two days' excretion.

with red corpuscles, and pulp cells were numerous only in residual islands remote from the sinusoids. The sinusoids presented an empty appearance except for adherent large white cells within the lumina. The red corpuscles were strikingly elongated and even filiform. Areas of coarse brown granules were observed in and near some of the coarser trabeculae and in the capsule.

This pigment gave the Prussian blue reaction for iron. There was no trace of finely granular pigment or of red cell phagocytosis.

Polychrome methylene blue and eosin preparations showed diffusely scattered cells resembling eosinophilic myelocytes. The red cells, which took a deep polychrome stain, were packed in largest numbers in the perisinusoidal zones, which were definitely delimited from the surrounding reticulum.

The tissue fixed in Zenker's solution cut badly because of great friability. However, several sections showed definitely that the red cells were normal in appearance and not elongated.

Stained smears were made of red cells triturated out of unblocked tissue fixed in 4 per cent formaldehyde. These red cells were all elongated, and many were crescentic.

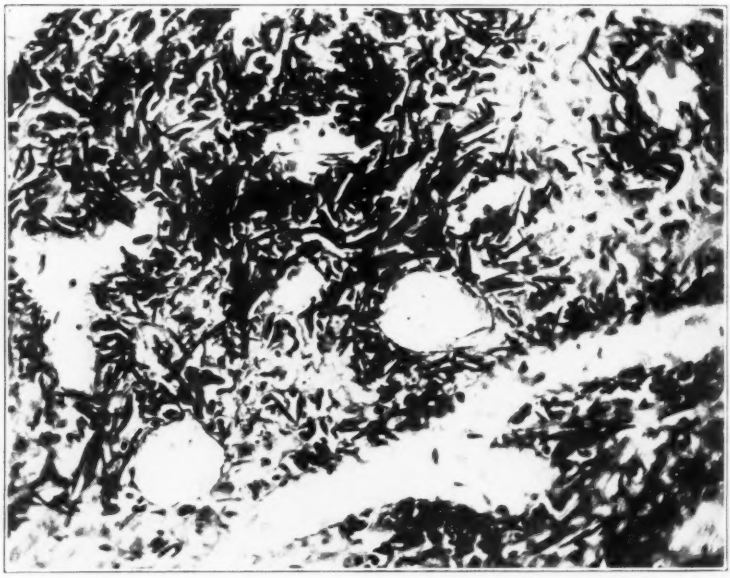

Fig. 3.—Section of the spleen stained by the Gram-Weigert method. The photomicrograph was made with light filtered to increase the contrast between the tissue and the red corpuscles, which were stained deep purple. The rod-shaped, intensely black objects represent elongated red corpuscles lying with their long axes in the plane of the section. The small round and elliptical black objects represent cross and oblique sections of elongated red corpuscles.

MECHANISM OF THE SICKLE DISTORTION

Neither our patient nor any member of his family had definite sickle cells in their circulating blood as far as we could determine by examination of stained or fresh wet smears. Our patient was shown to belong to the sickle cell anemia group by the demonstration of sickle cells in sealed wet smears kept at room temperature for twenty-four hours. The patient's mother and two of his sisters were shown to have "latent sickle cell anemia."

It is apparent from these facts, as well as from published reports, that the sickle cell is not a distorted cell produced as such by the erythropoietic tissue. On the other hand, it is indistinguishable from normal cells until acted on by unknown factors. In undertaking an investigation of these factors, we hoped to gain some insight into the nature of the fundamental defect of the cells and the mechanism of the production of anemia.

Previous experiments with sickle cells have yielded the following data:

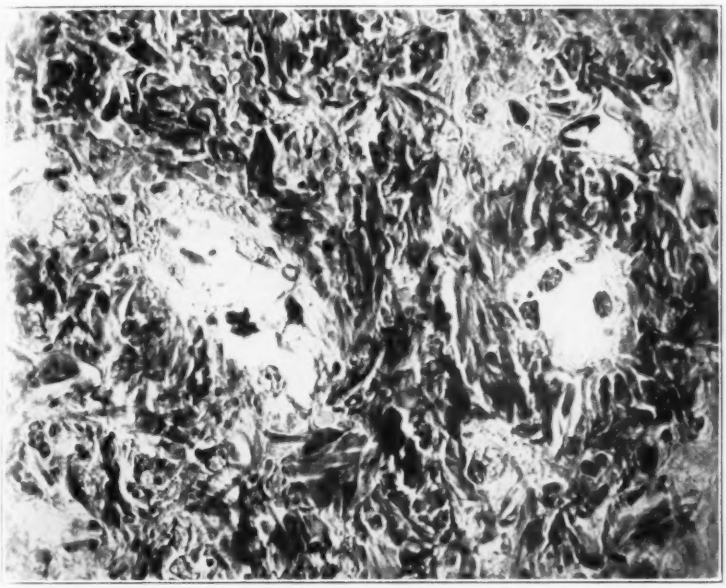

Fig. 4.—Section of the spleen stained with polychrome methylene blue and eosin, showing the concentration of the elongated red corpuscles in perisinusoidal zones which are more or less delimited from the surrounding reticulum. Large cells are shown adherent to the sinusoidal endothelium.

1. Sickle cell formation is retarded by cold and accelerated by heat.[6]

2. Sickle cell formation is independent of exposure to light, although return to spherical shape is more rapid in the dark.[7]

3. Serum is not essential for sickle cell formation, as the susceptible cells undergo the distortion in saline or citrate suspension.[17]

17. Huck, J. G. (footnote 7). Sydenstricker, Mulherin and Houseal reported that serum is essential. Their negative result cannot be as conclusive as the positive results obtained by Huck which were also confirmed by our own observations.

4. Susceptible cells, washed, become sickle cells in normal serum of the same blood type.[18]

5. Normal cells do not become sickle cells by the action of the serum of a person whose blood contains sickle cells.[18]

6. Bile pigment and bile salts accelerate sickle cell formation.[6]

7. Phagocytosis of sickle cells by large mononuclears occurs in vitro.[6]

8. Large mononuclears of a person whose blood contains sickle cells do not phagocytize normal red cells.[6]

9. Production of sickle cells is inconstant in preparations made under identical conditions.[19]

10. Red cells of tissue of affected subjects, fixed in formaldehyde, are sickle cells; when Zenker's solution is used, sickle cells are not present.[20]

In the course of preliminary experiments testing the effect of formaldehyde, we found that the patient's corpuscles sedimented by gravity from a neutral citrated saline suspension and drawn from the bottom of a serum tube with a pipet showed a considerable percentage of sickle cells. Samples drawn from the same suspension after agitation showed no sickle cells.

The most obvious difference between corpuscles which had settled at the bottom of a serum tube, and corpuscles which had recently been agitated was oxygen tension, if one assumes that the corpuscles and perhaps the leukocytes continued a metabolism which rendered their environment progressively poorer in oxygen. As the product of such an assumed metabolism would be carbon dioxide, we were obliged to take into consideration (1) the presence of carbon dioxide, (2) a shifting in the hydrogen ion concentration and (3) deprivation of oxygen.

Acting on these considerations, we arranged a series of experiments in which hanging drops of the corpuscle suspension, inverted over a gas chamber provided with inlet and outlet tubes, were observed with a 4 mm. objective. Various gases were passed through the chamber. Figure 6 shows in diagram the arrangement of the apparatus.

EFFECT OF VARIOUS GASES ON CORPUSCLES IN CITRATE-SALINE SUSPENSION

Carbon dioxide was passed through the gas chamber in a gentle stream after thorough saturation with water vapor at room temperature. Sickle formation of the patient's corpuscles occurred in less than two

18. Huck, J. G. (footnote 7), Sydenstricker (footnote 6).

19. Graham, G. S. (footnote 9), Anderson, H. B. (footnote 11). Also confirmed by our own observations.

20. Graham, G. S. (footnote 9). Also confirmed by our own observations.

minutes. Nitrous oxide and hydrogen each caused identical reactions in from two to five minutes. Nitrogen failed to cause sickle formation in the unaltered (neutral) suspension. Ethylene brought about perfect sickle production on its first trial. On subsequent trials, its effect on the corpuscles of the unaltered (neutral) suspension was inconstant.

Oxygen and carbon dioxide were found to be absent, as impurities, in samples of the aforementioned gases in amounts which could be detected by the Haldane gas analysis apparatus. All the gases were examined for the presence of volatile substances of acid nature by bubbling them through freshly distilled water for thirty minutes. A shift

Fig. 5.—Stained smear of the red blood corpuscles triturated out of spleen tissue fixed in formaldehyde.

in the hydrogen ion concentration of the water was not detected in any case by the colorimetric test.

REVERSIBILITY OF THE SICKLE CELL DISTORTION

The admission of pure oxygen to the gas chamber after the sickle cell formation by carbon dioxide instantly brought the cells to a discoid state. This reversal was equally prompt after exposure to all the gases causing sickle cell formation.

Carbon monoxide was passed through the gas chamber. The corpuscles in equilibrium with carbon monoxide retained their discoid contour. Passing carbon dioxide after exposure to carbon monoxide

brought about sickle cell formation in four minutes. A new exposure to carbon monoxide caused instant reversal to the discoid form. The experiment was repeated, starting with carbon dioxide, and a long series of reversals was carried out.

INTERPRETATION OF DATA SO FAR REPORTED

The most significant common property of carbon dioxide, hydrogen and nitrous oxide is that they contain no oxygen which is chemically available under the conditions of the experiment. The most obvious conclusion to be drawn from the fact that these three gases induced sickle cell formation is that the distortion occurs as a result of oxygen asphyxia of the susceptible corpuscles.

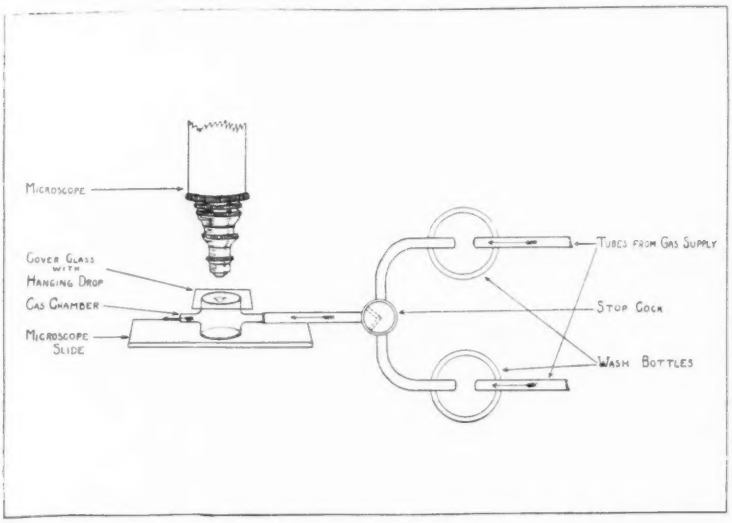

Fig. 6.—Arrangement of the apparatus used to test the effect of gases on red corpuscles.

The validity of this conclusion apparently is threatened by the fact that ethylene failed to induce sickle cell formation constantly, and that nitrogen failed entirely when the corpuscles were tested in the original neutral citrated saline suspension. That this discrepancy is only apparent will be shown in the section on the influence of hydrogen ion concentration on the asphyxia reaction.

The behavior of the corpuscles toward oxygen greatly strengthens the conclusion stated in the foregoing and shows that sickle cell formation is a reversible phenomenon depending on the oxygen tension of the medium in which the cells are suspended. In the presence of oxygen, the discoid form is stable; in its absence the distorted form is stable.

The behavior of the corpuscles toward carbon monoxide calls attention to a second property common to the gases causing sickle cell production. None of them forms as definite or as stable a compound with hemoglobin as do oxygen and carbon monoxide. The previously stated conclusion may therefore be expanded into the following hypothesis: Sickle cell formation is a reversible phenomenon depending on the free or combined state of the hemoglobin of the susceptible corpuscles. When the hemoglobin is in the combined state, the discoid form is stable; when in the uncombined state, the distorted form is stable.[21] The behavior of the susceptible corpuscles toward oxygen asphyxia thus constitutes a special application of a hypothesis correlating contour with the state of the hemoglobin.

DEGREE OF SENSITIVENESS TO OXYGEN

In all the foregoing experiments, pure gases were used to effect the reversal to discoid form. In order to determine the partial pressure of oxygen rendering the sickle form unstable, mixtures of carbon dioxide and oxygen were prepared, passed through the gas chamber and then analyzed in the Haldane apparatus.

A mixture proving by analysis to be 6.4 per cent oxygen failed to cause any typical sickle cell production during ten minutes of exposure. A mixture wtih 3.5 per cent oxygen caused about 50 per cent of the corpuscles to assume the sickle form in five minutes and about 90 per cent of them in eight minutes.

We concluded that the maximum concentration of oxygen in which the sickle form is stable is about 6 per cent, which corresponds to a partial pressure of 45 mm. of mercury.

ASPHYXIA REACTION OF CORPUSCLES OF MEMBERS OF PATIENT'S FAMILY AND OF CONTROLS

The corpuscles of the patient's mother and of two of his sisters showed the sickle reaction when tested in neutral citrated saline suspension with carbon dioxide. The corpuscles of the two sisters showed the sickle reaction when tested in neutral suspension with hydrogen and nitrious oxide.

These relatives of the patient were apparently well. None had splenomegaly or anemia, and none showed sickle cells in stained smears or wet smear cultures; yet their red corpuscles were clearly demon-

21. A possible objection to our hypothesis may be based on the known relative stability of carbonyl hemoglobin. It must be remembered, however, that the corpuscles were in a thin film of suspension, and that the carbon dioxide was moving in a stream, carrying out of the gas chamber all traces of gaseous carbon monoxide. The conditions were thus proper for the propressive completion of an equilibrium reaction in accordance with the law of mass action.

strated to have the fundamental defect which renders them susceptible to distortion when subected to oxygen asphysia.

That the sickle distortion as an asphyxia reaction is specific for the corpuscles of certain family groups of negroes is indicated by the negative results obtained on examining the corpuscles of numerous controls by the technic which has been described. The negative controls consisted of the father and paternal grandmother of the patient, negro children in the hospital with ailments unrelated to anemia, a patient with pernicious anemia and numerous healthy white subjects.

The technic used in the foregoing experiments, simplified by omitting provision for two gases, is proposed as a reliable and rapid diagnostic test for sickle cell anemia. A drop of the subject's blood in physiologic sodium chloride, a gas chamber, a microscope and a cylinder of carbon dioxide, which is available in most hospitals, are all that is necessary. Reversibility of the distortion can readily be demonstrated by admitting a little air to the hanging drop.

INFLUENCE OF HYDROGEN ION CONCENTRATION IN THE ASPHYXIA REACTION

In a previous section it was stated that the patient's corpuscles in neutral suspension failed to change to a sickle cell form when tested with nitrogen, and that they sometimes failed when tested with ethylene. Reference to table 3 will show that in a neutral suspension, the corpus-

TABLE 3.—*Reactions of Corpuscles of Various Persons to Pure Gases in Equilibrium with Hanging Drops of Corpuscle Suspension*

Subject	Carbon Dioxide, Neutral	Hydrogen, Neutral	Nitrous Oxide		Ethylene		Nitrogen	
			Neutral	Acid	Neutral	Acid	Neutral	Acid
Patient	+*	+	+	+	±	+	—	+
Mother	+	..	—	+	—	+	..	..
Sister, 2 years	+	+	+	+	—	+	..	..
Sister, 9 months	+	+	+	+	—	+	..	..
Father	—	..	—	—	—	—	..	..
Paternal grandmother	—	..	—	—	—	—	..	..
Normals	—	—	—	—	—	—	..	..
Pernicious anemia	—	—	—	—	—	—	..	..

* The sign + means that sickling occurred; —, that sickling did not occur; ±, that sickling occurred on some trials and did not occur on others; a blank (..) space, that no test was made. In the columns headed "Neutral" are the reactions of the corpuscles in an approximately neutral citrated 0.8 per cent sodium chloride solution. In the columns headed "Acid" are the reactions of corpuscles suspended in the same medium altered by the addition of an equal volume of buffer solution of p_H 6.8.

cles of the patient's mother and of two of his sisters failed to assume a sickle cell shape with ethylene, and that in neutral suspension the corpuscles of the mother failed to assume this form with nitrous oxide.

This anomalous behavior of susceptible corpuscles toward oxygen-free gases was taken to indicate the participation of an additional factor in the reaction. The fact that carbon dioxide, the only gas of the series which altered the hydrogen ion concentration of the aqueous medium.

invariably caused sickle cell formation led to the suspicion that certain conditions of hydrogen ion concentration are prerequisite to the reaction.

Portions of the corpuscle suspensions of the patient, the mother and the two sisters were mixed with equal volumes of a buffer solution of p_H 6.8. These acidified suspensions were then tested with the aforementioned gases which had produced no sickle cell formation in the corresponding neutral suspensions. Sickle cells occurred in every instance within five minutes after starting the stream of gas, and was constant on repeated trials.

Previous acidification was not necessary in the reaction of the corpuscles of the patient with hydrogen and nitrous oxide; neither was it necessary in the reaction of the corpuscles of the two sisters with nitrous oxide, nor in the reaction of the patient's corpuscles with ethylene on occasional trials.

As these inconsistencies were neither constant nor significantly associated with any other properties of the gases, it was considered that they arose from differences in the sensitiveness to asphyxia of corpuscles from various persons and from accidental shifts in the hydrogen ion concentration.

RANGE OF HYDROGEN ION CONCENTRATIONS WITHIN WHICH SICKLE CELL FORMATION OCCURS IN ASPHYXIA

As a check on the results already obtained, it was considered advisable to determine the limits of hydrogen ion concentration within which sickle cell formation occurs in oxygen asphyxia. Ethylene was used in this series of experiments because more often than any of the other gases it failed to cause sickle cell formation of susceptible corpuscles in neutral suspension.

Two methods of altering the hydrogen ion concentration of the suspension were employed: First, equal drops of the citrated saline suspension and the buffer solution were mixed on cover slips which were then inverted over the gas chamber. After several minutes of observation to make sure that sickle cell formation was not induced by shift of the hydrogen ion concentration alone, the ethylene was turned on. Second, one-hundredth cubic centimeter portions of a thick suspension of washed corpuscles were mixed in small test tubes with 1 cc. portions of buffer solutions. Small drops of these suspensions were then mounted and treated as before.

We found it possible to measure roughly the relative suitability of the various suspensions to form sickle cells by estimating in percentage the extent of the reactions as judged by the numbers of corpuscles that had assumed the sickle cell shape and by the completeness of the distortion of individual corpuscles at the expiration of five minute periods.

Sickle cell formation generally occurred more quickly at the edges of the drops, and in the less suitable solutions, only there. In two series of trials with the patient's corpuscles, by the method of suspension, we kept separate data of the reaction at the edge and at the center.

The results shown graphically in figures 7 and 8 are consistent in general. From the point of beginning hemolysis in the more acid suspensions, the ability to undergo sickle cell formation rapidly increases with decreasing acidity to a maximum which is maintained over a wide range, and begins rapidly to decrease with increasing alkalinity at a point not far from neutrality.

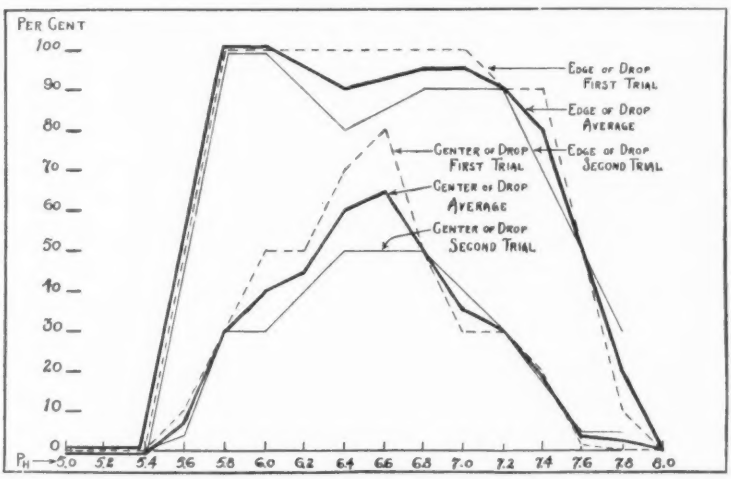

Fig. 7.—Completeness of sickle cell formation of the patient's corpuscles, estimated in percentage, at various hydrogen ion concentrations. Ethylene was used to exclude air. The determinations were made on suspensions prepared from washed corpuscles and the various buffer solutions.

The fact that the patient's corpuscles assumed a sickle cell shape in alkaline suspensions up to p_H 7.8, whereas the corpuscles of his symptomless relatives did not assume a sickle cell shape except in suspensions on the acid side of p_H 7.4, is consistent with the greater susceptibility of the patient's corpuscles as shown by wet smear cultures. However, the patient's corpuscles were washed free from plasma, whereas those of the relatives were not. Furthermore, the physiologic sodium chloride used for the washing was later shown to have a p_H 6.8. When the patient's blood was added directly to the buffer solutions, results were obtained in fair agreement with those for the relatives, as shown by curve A, figure 8.

Great numerical accuracy is not claimed for these determinations. The actual hydrogen ion concentration of the liquid phase of the altered

suspensions was not measured. Dilution of the buffer solutions as well as the addition of blood proteins and salts must have changed the hyrogen ion concentration from its previously measured value. The stream of ethylene also doubtless altered the hydrogen ion concentration by sweeping out of the gas chamber all traces of carbon dioxide, thus causing a shift in the whole train of equilibria among gaseous carbon dioxide, dissolved carbon dioxide, carbonic acid and sodium bicarbonate.

However, this series of determinations proves that acidification up to the point of beginning hemolysis, favors the sickle reaction. Since an increase in hydrogen ion concentration favors the dissociation of

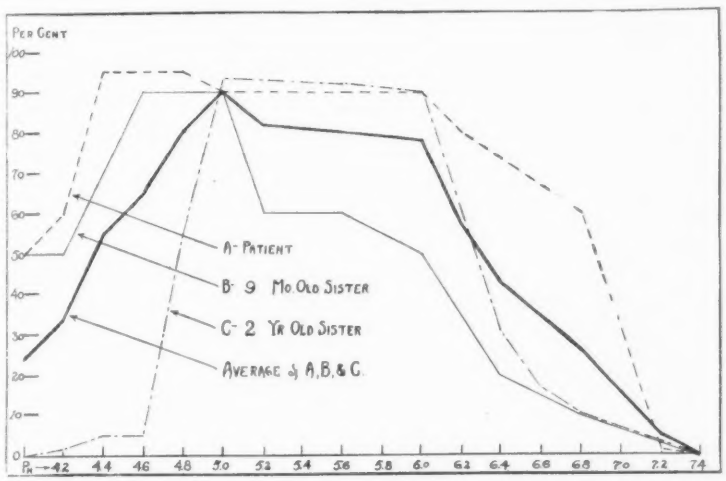

Fig. 8.—Completeness of sickle cell formation of susceptible corpuscles, estimated in percentage, at various hydrogen ion concentrations. Ethylene was used to exclude air. The corpuscles were not washed. Curves *B* and *C* represent averages of (*1*) determinations on drops prepared by mixing corpuscle suspension with buffer solution on cover slips, and (*2*) determinations on suspensions prepared by adding citrated blood to buffer solution. Curve *A* represents the averages of two series of determinations on drops prepared as designated above by (*1*).

oxyhemoglobin, the effect of hydrogen ion concentration on the sickle reaction is precisely what would be predicted from our hypothesis relating the distortion to the state of the hemoglobin within the corpuscles.

SHIFT OF HYDROGEN ION CONCENTRATION ALONE DOES NOT CAUSE SICKLE CELL FORMATION

In the course of the experiments just reported, the effect of acid and alkaline solutions on the corpuscles prior to the displacement of air from

the gas chamber was observed many times, and in no instance did sickle cell formation occur.

As a further control, small drops of the suspension of the patient's corpuscles were mixed with large drops of buffer solutions of p_H varying by 0.2 from p_H 4.4 to p_H 8. After thorough mixing of the drops, cover slips were applied and sealed with petrolatum. The preparations were examined at intervals for two hours. No sickle cells appeared in any of the smears.

SICKLE CELL FORMATION INDEPENDENT OF OSMOTIC PRESSURE

It became necessary to investigate the effect of osmotic pressure on the sickle cell formation phenomenon because the buffer solutions, prepared by the method of Clark and Lubs [22] differed in concentration of salt.

Three series of experiments were performed. In each, the patient's washed corpuscles were added to the specially prepared solutions and then tested for the sickle reaction with ethylene.

In the first series, the hydrogen ion concentration of 0.9 per cent sodium chloride solution was altered by adding traces of acetic acid and sodium hydroxide to make a series corresponding to the buffer solutions. Sickle cell formation occurred over a range of hydrogen ion concentrations roughly comparable to those found adequate when buffers were employed.

In the second, enormous changes were made in the osmotic pressure of a buffer solution of p_H 5.6 by diluting it with freshly distilled water and by adding 10 per cent sodium chloride solution. The solutions thus prepared formed a series of practically constant p_H and of sodium chloride concentrations varying from 0.2 per cent to 2.2 per cent by 0.2 per cent intervals. Sickle cell formation occurred in every case from the point of beginning hemolysis in the more dilute solutions up to extreme crenation in the more concentrated.

In the third, solutions of sodium chloride were prepared varying in concentration from 0.5 per cent to 1.2 per cent by intervals of 0.05 per cent. Each solution was tested colorimetrically before use, and the hydrogen ion concentrations were all found to lie between p_H 7.2 and p_H 7.4. Sickle cell formation occurred in all the dilutions with the exception of those causing hemolysis.

These results indicate that osmotic pressure has no influence on the sickle cell formation phenomenon between the points of beginning hemolysis and extreme crenation. The failure of corpuscles which have lost their hemoglobin to undergo sickle cell formation is consistent with our hypothesis relating the distortion to the hemoglobin.

22. Clark and Lubs, referred to by Clark, W. M.: The Determination of Hydrogen Ions, Baltimore, Williams & Wilkins Company, 1920, p. 75.

SICKLE SHAPE DISTORTION OF A CORPUSCLE

The first change noted in a corpuscle eventually forming a perfect sickle is a subtle change in the refractility of the erythroplasm. Next, a blotchy opacity appears at one side in the very rim of the biconcave disk and extends across the disk in radiating lines. This parietal opaque focus grows thicker, and the opposite side of the disk becomes thinner until it is scarcely visible. Meanwhile, the side on which the opacity first appeared decreases its curvature, and the thinned out opposite side is stretched from horn to horn of the resulting half moon. As the change progresses, the horns become longer, and the attenuated side becomes concave, until a slender crescent is formed. Generally, before this end stage is reached, several sharp processes are extruded from the convex side, producing a crisp brushlike appearance.

When oxygen is admitted to the sickle cell, the resumption of the circular form is startling in its abruptness. The sharp processes are retracted, the concave border swells out, the curvature of the convex border increases, and the mottled opacity is replaced by the normal degree of transparency.

THEORY OF THE PATHOGENESIS OF THE HEMOLYTIC ANEMIA OF PERSONS WHOSE BLOOD CONTAINS SICKLE CELLS

It is impossible for us to conceive of the sickle cell trait as a disease in the ordinary sense. It is hereditary and is compatible with good health and long life. However, it predisposes those affected by it to hemolytic anemia. It is possible that the mechanism of sickle cell formation in vitro, established by our experiments, may be related to the pathogenesis of this anemia.

It is reasonable to suppose that sickle cells are formed in the body of an affected person wherever the oxygen tension and hydrogen ion concentration are such as to render the distorted form of the corpuscles stable. It is also probable that a condition of general anoxemia would induce the formation of sickle cells in vivo.

If corpuscles are permanently damaged by the sickle distortion or are rendered more susceptible to hemolytic influences while distorted, anemia would be the inevitable result of anoxemia.[23] Vigorous phagocytosis of sickle cells in vitro has been observed by Sydenstricker, and large mononuclears with phagocytosed corpuscles have been observed repeatedly in stained smears of the circulating blood of patients with sickle cell anemia.

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23. Cooley, T. B., and Lee, Pearl: The Sickle Cell Phenomenon, Am. J. Dis. Child. **32**:334 (Sept.) 1926. The authors report that sickle cells undergo hemolysis quickly at incubator temperature whereas normally shaped cells of the same subject do not.

Anoxemia, then might constitute at least one of the unknown factors hypothecated by Sydenstricker [8] and Graham [9] which make the difference between latent sickle cell anemia and active sickle cell anemia. According to this view, if the sickle cell trait were universal, pulmonary and cardiac disease reducing oxygen tension in the body invariably would be attended by anemia and jaundice.

It is admitted that this theory rests in part on assumptions. However, there is considerable evidence in the reported case histories to support it. Table 4, summarizing the essential data of the thirteen cases reported in detail, shows that evidence of pulmonary pathologic change occurred in 60 per cent of the patients and evidence of cardiac pathologic change in 100 per cent. In one of Huck's cases and in our case a preliminary diagnosis of mitral insufficiency was made. Huck's other

TABLE 4.—*Summary of Essential Clinical and Laboratory Data from Reported Patients with Active Sickle Cell Anemia**

	Case	Green Sclera	Joint Pain	Ulcer of Leg	Abdominal Pain	Liver Enlarged	Spleen Enlarged	Pathologic Change of Lung	Heart Enlarged	Heart Murmur	Improved by Rest	Albuminuria	Casts	Bile in Urine	Van den Bergh Test Direct	Van den Bergh Test Indirect and Units of Serum Bilirubin	Increased Urine Urobilin	Limits of Hemolysis Sodium Chloride Concentration, Percentage	Reticulocytes Percentage of Red Cells
Herrick	1	+	+	+	+	+	+	+	+	+	+	+	+	+	..	..	..	.....	..
Washburn	2	+	+	+	+	+	+	+	+	+	+	+	+	+	..	..	..	.....	..
Cook and Myer	3	+	+	—	+	+	+	+	+	+	+	+	+	+	..	..	..	0.53-0.25	..
Mason	4	+	+	—	+	+	+	+	+	+	+	+	+	+	..	..	..	0.30-0.18	23
Sydenstricker et al.	5	+	+	—	+	+	+	+	+	+	+	+	+	+	..	..	+	0.45-0.26	24
Sydenstricker et al.	6	+	+	—	+	+	+	+	+	+	+	+	+	+	..	..	+	0.45-0.24	34
Huck	7	+	+	+	+	+	+	+	+	+	†	+	+	+	..	4.4	+	.....	30
Huck	8	+	+	+	..	+	+	±	+	+	†	+	+	±	..	..	+	.....	35
Graham	9	+	+	+	+	+	+	†	+	+	†	+	+	±	—	+	+	0.34-0.16	30
Moser and Shaw	10	+	..	—	+	+	+	..	+	+	..	+	+	..	±	...	..	0.28-0.20	..
Anderson	11	+	+	+	—	+	+	..	+	+	..	+	+	..	±	8.0	+	0.35-0.20	6
Hamilton	12	+	+	+	+	+	+	..	+	..	..	+	+	..	±	+	+	0.42-0.28	9
Browne	13	+	+	+	—	+	—	+	..	+	..	—	—	—	..	10.0	..	0.32-0.22	..
Hahn and Bierman	14	+	+	—	+	+	+	+	+	+	—	—	—	—	—	2.9	+	0.32-0.20	19

* Case 14 is presented in this article. The report of two cases by Cooley and Lee, footnote 23, appeared too recently for inclusion in this table. The sign + means that the phenomenon designated in the column heading was present; —, that it was absent; ±, that its presence was doubtful; a blank (..) space, that it was not reported by the author; †, that death resulted. The figures for serum bilirubin as reported in the literature were reduced to "units," as defined by Ravdin: An Estimation of the Clinical Value of the van den Bergh Test, Am. J. M. Sc. **169**: 850 (June) 1925, for convenience of comparison.

patient died of pulmonary tuberculosis. It is not more hazardous to assume that the instances of cardiac hyperthrophy and loud systolic murmurs transmitted to the axilla were caused by primary cardiac pathologic change than it is to assume that they were altogether secondary to the anemia. It is noteworthy that several of the patients in the cases reported were greatly improved by rest in bed together with drug therapy which can hardly be supposed to have had any specific influence on the course of the disease.

The spleen cannot be left out of account in an explanation of sickle cell hemolytic anemia. Our experience indicates that the spleen plays an important part in the mechanism of the excessive blood destruction. After splenectomy was performed, startling reduction of the previously excessive rate of hemolysis was demonstrated by the chemical studies on the blood and excreta, by the hematologic studies and by the clinical improvement.

To account for this result we are not inclined to invoke the questionable hypothesis of "hypersplenism." The pathologic changes in the spleen and the changes reported by Sydenstricker suggest that the organ is more injured than hypertrophied. It is enough to assume that the spleen merely performs its usual function in sickle cell anemia, that of capturing and destroying damaged or abnormal red blood corpuscles, and that in performing this office, it is damaged itself. Splenectomy, then, is beneficial because it frees the patient from a hemoclastic organ which is, under the circumstances, too discriminating. Sickle cell anemia may be a state in which damaged or sickle shaped red blood corpuscles are preferable to none at all.

One fact in our case demonstrates that the influence of the spleen is not the cause of sickle cell formation nor of the fundamental abnormality of the erythropoietic tissue. Our patient still shows the sickle cell trait four months after splenectomy.

Sydenstricker, by inference, attributes splenomegaly in sickle cell anemia to coincident or previous malaria. Our patient presented no evidence or history of malaria, and malaria is uncommon in Indianapolis; yet his spleen was exceedingly large. It is likely that the size of the spleen in patients with sickle cell anemia depends on the stage of the disease. Although the spleen plays an active, but probably secondary, rôle in corpuscle destruction, it is, nevertheless, greatly injured by its long continued over-use, and completes its life history as an atrophic remnant of a once enlarged organ.

As regards the cause of "active sickle cell anemia," the specific characteristics of the condition are sufficiently explained by the underlying predisposition which is unique in pathology. It is unnecessary to assume an additional specific etiologic agent. Indeed, all the known facts of the disease, the geographic distribution, the restriction to negroes, the sporadic incidence of the active disease among large groups of susceptible but unaffected persons, the relatively small number of patients with active anemia in contrast with the large number of susceptible persons, makes such an assumption extremely hazardous.

In our view, it is highly desirable to distinguish sharply between the hereditary predisposition to form sickle cells and the disease which depends on it. The term "latent sickle cell anemia" is unsatisfactory

as applied to clinically healthy persons who have no demonstrable anemia. Furthermore, the term is awkward. For "sickle cell" we propose drepanocyte;"[24] for the inherited trait, "drepanocytemia;" for the active disease, drepanocytic anemia."

CONCLUSIONS

1. The red corpuscles of persons with the "sickle cell trait" are transformed into sickle cells in vitro as a result of asphyxia. The transformation takes place when the oxygen tension falls below a partial pressure of 45 mm. of mercury, provided the hydrogen ion concentration is within certain limits, probably always on the acid side of p_H 7.4.

2. The sickle distortion is a reversible phenomenon. Oxygen and carbon monoxide induce restoration of the discoid form.

3. All of the facts relating to sickle cells are consistent with a hypothesis that the sickle form is stable when the hemoglobin is dissociated, and that the discoid form is stable when the hemoglobin is combined.

4. The influence of hydrogen ion concentration in the sickle reaction is probably related to its influence on the dissociation of oxyhemoglobin.

5. Sickle cell formation in vivo is probably induced or increased by anoxemia.

6. Disease of the heart and lungs probably plays an important rôle in causing excessive hemolysis when it occurs in persons with the sickle cell trait.

7. Reasons exist for believing that the only specific cause for active sickle cell anemia is the unique hereditary anomaly of the red corpuscles which predisposes to it.

8. A patient with active cell anemia is reported as having been greatly improved by splenectomy.

9. The spleen probably plays a secondary rôle in the excessive hemolysis of active sickle cell anemia and is damaged in the process, passing through stages of enlargement to ultimate atrophy.

10. The influence of the spleen is not the cause of the sickle cell trait.

11. A laboratory test for the sickle cell trait is described.

12. The terms "drepanocyte," "drepanocytemia," and drepanocytic-anemia" are proposed to replace, respectively, the terms "sickle cell," "latent sickle cell anemia" and "active sickle cell anemia."

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24. The word "drepanocyte" is derived from the Greek word δρεπάνη, sickle.

BASAL METABOLISM IN CHRONIC MYELOGENOUS LEUKEMIA *

MATTHEW C. RIDDLE, M.D.
AND
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BOSTON

The basal metabolism in untreated patients with leukemia is characteristically elevated. No other afebrile disease, with the exception of hyperthyroidism, exhibits such a constant increase in heat production by the body. The high level of the metabolism in hyperthyroidism appears to be associated definitely with an increased or altered secretion of the thyroid gland, but the underlying factor or factors which account for this phenomenon in the leukemias still remain a matter for speculation and investigation. Many studies have been made concerning the value of determining the basal metabolic rate in hyperthyroidism, its use as an index of the course of the disease and its relation to the diagnosis and prognosis. On the contrary, observations on the basal metabolism in the leukemias have been much less frequent, and as a large number of patients with this disease are encountered at the Collis P. Huntington Memorial Hospital and at the Peter Bent Brigham Hospital, it was thought that an intensive study of the basal metabolism in this condition might be of importance.

The literature on the basal metabolism in leukemia has been reviewed by DuBois,[1] McCann,[2] Boothby and Sandiford,[3] and by Murphy, Means and Aub.[4] Early investigators reported conflicting evidence as to the rate of oxygen metabolism in patients who had leukemia. In 1911, Grafe [5] first demonstrated the marked increase in the basal metabolism

* From the Medical Clinics of the Collis P. Huntington Memorial Hospital of Harvard University and of the Peter Bent Brigham Hospital.

* This paper is no. 64 of a series of studies in metabolism from the Harvard Medical School and allied hospitals. The expenses of this investigation have been defrayed (in part) by a grant from the Proctor Fund of the Harvard Medical School for the study of chronic diseases.

1. DuBois, E. F.: Basal Metabolism in Health and Disease, ed. 1, Philadelphia and New York, Lea and Febiger, 1924, p. 300.

2. McCann, W. S.: Colorimetry in Medicine, Medicine **3**:1, 1924.

3. Boothby, W. M., and Sandiford, I.: Basal Metabolism, Physiol. Rev. **4**:69 (Jan.) 1924.

4. Murphy, J. B.; Means, J. H., and Aub, J. C.: The Effect of Roentgen-Ray and Radium Therapy on the Metabolism of a Patient with Lymphatic Leukemia, Arch. Int. Med. **19**:890 (May) 1917.

5. Grafe, E.: Die Stiegerung des Stoffweschels bei chronischer Leukämie und ihre Ursachen, Deutsches Arch. f. klin. Med. **102**:406, 1911; Die pathologische Physiologie des Gesamststoff- und Kraftwechsels bei der Ernahrung des Menschen, Ergebn. d. Physiol. **21**:464, 1923.

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in both chronic lymphatic and myelogenous leukemia. He suggested from experiments on blood in vitro that the rise in the basal metabolism could be explained, in part at least, by an actual increase in the oxygen consumption by the exceedingly large number of white blood cells. He attributed the additional increase, not accounted for on this basis, to the increased use of oxygen by the excessive number of white blood cells stored in the body elsewhere than in the circulating blood, and growing abnormally in the marrow and often in other tissues.[5] Murphy, Means, and Aub,[4] studied the metabolism in a man with chronic lymphatic leukemia before and after treatment with radon and the roentgen rays. They observed that the basal metabolism, which was greatly elevated before treatment, decreased only slightly with irradiation, although the white blood count decreased markedly. Gunderson,[6] in a study of nineteen patients who had chronic myelogenous leukemia on whom sixty-two determinations of the basal metabolism were obtained, observed that the rise in basal metabolism appeared to be associated more closely with the number of immature white blood cells than with the total number of white blood cells. Striking exceptions were noticed, however, in which there was no correlation between the basal metabolism and the white blood count or the percentage of immature cells. McAlpin and Sanger [7] studied the basal metabolism in seven patients with chronic lymphatic leukemia, eight patients with chronic myelogenous leukemia and one patient of "mixed type" over considerable periods of time. They concluded that the metabolism rose and fell with the white cell count, and that after irradiation with roentgen rays, the basal metabolism often fell to within normal limits, rising once more as the white blood cells again increased in number. In their opinion the rise in the metabolism often preceded the increase of white blood cells and was consequently of value as one of the indications for further treatment.

In the present study an attempt has been made to determine the relationship between the alterations in the blood cells and the level of the basal metabolism. Furthermore, the significance of the latter has been considered in relation to the diagnosis and prognosis of chronic myelogenous leukemia. Two methods were used in studying the available material. First, a statistical study was made of thirty-six patients with chronic myelogenous leukemia in all phases of the disease, in whom the basal metabolism had been determined 272 [8] times. Three of the patients on whom five determinations were made will be considered

6. Gunderson, A. H.: The Basal Metabolism in Myelogenous Leukemia and Its Relation to the Blood Findings, Boston M. & S. J. **185**:785 (Dec. 29) 1921.

7. McAlpin, K. R., and Sanger, B. J.: Blood Counts and Basal Metabolism of Leukemias Under Roentgen-Ray Treatment, Am. J. M. Sc. **167**:29 (Jan.) 1924.

8. Approximately one half of the data were collected prior to this study, owing to its anticipation.

separately on account of the unusual character of the blood picture. Second, intensive studies were made on five patients with chronic myelogenous leukemia in the form of daily observations of the metabolism and blood picture.

All determinations of the basal metabolic rate were made on the Roth modification of the Collins-Benedict [9] apparatus, usually by the graphic method. The customary cautions were observed to insure accuracy of the determinations. The basal metabolic rate was calculated from the standards of Aub and DuBois,[10] using the DuBois and DuBois [11] formula for surface area. Differential counts of the blood cells were made on at least 200 cells.

A STATISTICAL STUDY MADE ON TWO HUNDRED AND SIXTY-SEVEN DETERMINATIONS OF THE BASAL METABOLIC RATE IN CHRONIC MYELOGENOUS LEUKEMIA

In practically all instances the blood examinations were made at the same time as the basal metabolism determinations. From a statistical standpoint there is a possible criticism as the levels of the basal metabolism determinations were not uniformly distributed for its entire pathologic range; a large proportion were for the lower values and a smaller number for the higher ones. This factor was unavoidable and gives a slightly erroneous picture unless this fact is considered when deductions are made from the figures. Moreover, one patient (case 1) provided 104 of the total number of determinations, a fact which, however, appeared not to influence greatly the character of the averages obtained. A few other patients likewise contributed a considerable amount of the data concerning the basal metabolism, but a majority furnished three or four determinations.

DEGREE OF ELEVATION OF THE BASAL METABOLIC RATE

The impression is gained from the literature that low or normal values are exceptional in chronic myelogenous leukemia, and that without treatment high values are the rule. Table 1 gives the degree of elevation of the basal metabolism in sixty-one determinations on twenty-five patients with chronic myelogenous leukemia collected from the literature in which the results were suitable for comparison with the 267 determinations on our own thirty-three patients. The highest value for the

9. Roth, P.: Modification of Apparatus and Improved Technic Adaptable to the Benedict Type of Respiration Apparatus, Boston M. & S. J. **186**:457 (April 6) 1922.

10. Aub, J. C., and DuBois, E. F.: The Basal Metabolism in Old Men, Arch. Int. Med. **19**:823 (May) 1917.

11. DuBois, D., and DuBois, E. F.: A Formula to Estimate the Approximate Surface Area if Height and Weight Be Known, Arch. Int. Med. **17**:863 (June) 1916.

basal metabolic rate in chronic myelogenous leukemia recorded in the literature is plus 80.[6] In our series a similar value of plus 81 was found in an untreated patient. No hitherto reported estimations are lower than minus 10, although in our series there were four determinations below this figure; of these the lowest was minus 16. These low values, however, occurred in patients who had recently been treated with roentgen rays. Boothby and Sandiford[3] found that 27 per cent of fifteen patients with myelogenous or lymphatic leukemia had a metabolism of from minus 10 to plus 10; 7 per cent between plus 11 and plus 15; 6 per cent between plus 15 and plus 20; and 60 per cent over plus 20. Of the total number of our determination, 123 or 46.1 per cent were within normal limits of variation; 4 or 1.5 per cent

TABLE 1.—*The Degree of Elevation of the Basal Metabolism in Chronic Myelogenous Leukemia*

Range of Basal Metabolism	No. Estimations Recorded in Literature*	No. Estimations, Authors' Cases
−20 to −11	0	4
−10 to − 1	0	53
0 to + 9	5	70
+10 to +19	12	51
+20 to +29	14	43
+30 to +39	10	13
+40 to +49	7	12
+50 to +59	9	6
+60 to +69	0	9
+70 to +79	3	5
+80 to +89	1	1
Total	61	267

* These figures are taken from the cases collected by Murphy, Means and Aub from the literature and from the estimations made by Gunderson. In the former, Meeh's formula for surface area and the Gephart and DuBois standards for normal are used. In the latter, DuBois and DuBois formula for surface area and the Aub and DuBois standards for normal are used.

were below the normal limits of variation (minus 10); and 140 or 52.4 per cent were above the upper normal limit (plus 10). In only 46 determinations, or 17.2 per cent, was the metabolism elevated above plus 30. Untreated patients showed without exception an elevation of the basal metabolism above plus 10, and the majority of the determinations in such patients was between plus 20 and plus 30. Gunderson,[6] who also found a large proportion of low values, properly attributed these to recent therapeutic irradiation. The high percentage of low figures in our series of patients is doubtless due to the fact that many of the estimations were made after intensive roentgen-ray or radon treatment when the patients had been without symptoms for some weeks.

The relation of treatment to the height of the metabolism is, therefore, of importance. Table 2 shows the frequency distribution of 250 estimations of the basal metabolism in relation to the number of days since the last treatment. It is important to emphasize that after recent treatment, the metabolism falls rapidly if the slight transient rise

which occurs within the first few days following treatment is disregarded. A few patients proved to be exceptions to this statement, and they are designated in table 2 by an asterisk which indicates that they were in the terminal stages of the disease and, therefore, were not responsive to treatment. The effect of treatment on the metabolism and the duration of its beneficial effect can be deduced from table 2. As will be shown later, the immediate effect of irradiation on the basal metabolism in many instances is to produce a transitory rise usually lasting two or three days. Subsequently the metabolism usually falls, the extent of the decrease depending on the amount of treatment and on the condition of the patient. The absence of high values for a period of about ninety days following the three day period immediately subsequent to treatment indicates the decrease resulting from irradiation. The fact that

TABLE 2.—*The Frequency Distribution of the Basal Metabolism in Relation to the Number of Days Since the Last Roentgen-Ray or Radon Irradiation*

No. of Days Since Last Treatment	Basal Metabolic Rate (Number of Estimations)											
	−20 to −11	−10 to −1	0 to +9	+10 to +19	+20 to +29	+29 to +39	+40 to +49	+50 to +59	+60 to +69	+70 to +79	+80 to +89	Total
Untreated	..	..	..	6	13	1	..	5	..	..	1	26
1 to 3	..	9	22	16	18	3	7	1	4	4	..	84
4 to 10	2	20	27	5	1	..	..	..	..	..	..	55
11 to 30	1	21	8	3	..	..	1*	..	..	..	..	34
31 to 50	..	..	..	1	..	..	..	..	1*	..	..	2
51 to 70	..	..	1	1	1*	1*	..	..	..	..	..	4
71 to 90	..	1	1	..	2*	1*	..	..	2*	..	..	7
91 to 100	..	1	..	1	1	1*	..	..	1*	..	..	5
101 to 150	1	..	3	2	1	5	2	..	..	..	..	14
150 +	..	..	4	10	4	..	1	..	..	..	..	19
Total	4	52	66	45	41	12	11	6	8	4	1	250

* Determinations made on patients shortly before death.

after ninety days high values of the basal metabolic rate begin to reappear suggests the duration of the effect from irradiation. The beneficial result on the metabolism frequently persists much longer, especially in patients who are treated in the early stages of the disease.

Many of the estimations shown in table 2 in the three day period following treatment were within normal limits. The presence of these low figures is attributed to a summation of the effects of repeated and frequent exposures to the roentgen ray prior to the final exposure which was used to estimate the number of days since treatment. In these estimations there is no transient rise following irradiation which may be associated with the fact that the metabolism was already at a low level. The metabolism does not always fall subsequent to therapy. If such is the case, this usually indicates an active or terminal stage of the disease when the metabolism is at a high level, and the roentgen-ray exposures may have little or no effect on the metabolism or the condition

of the patient. High estimations, therefore, in patients who have been treated within ninety days and not as recently as three days, suggest a poor prognosis.

RELATION BETWEEN BASAL METABOLISM AND BODY TEMPERATURE

The advanced stage of chronic myelogenous leukemia is usually accompanied by fever, which in itself adds an increment to the already elevated metabolism. In 206 observations on the basal metabolism in which accurate information concerning the temperature of the patient was recorded, a buccal temperature above normal was found in twenty-one patients or in 10 per cent of the total number. Fever was usually slight when it was present, but in two patients the temperature was as high as 102 F. In all instances in which the temperature was distinctly elevated, the patients were in poor condition, and the metabolism greatly

TABLE 3.—*The Relation of the Average Pulse Rate to the Degree of Elevation of the Metabolism in Myelogenous Leukemia*

Number of Estimations	Range Basal Metabolic Rate	Average Basal Metabolic Rate	Average Actual Pulse	Range of Actual Pulse	Average Basal Pulse in Percentage*	Range of Basal Pulse in Percentage
4	—20 to —11	—14	56	51 to 60	89	85 to 93
53	—10 to — 1	— 5	62	54 to 90	101	90 to 129
66	0 to + 9	+ 5	64	52 to 91	106	87 to 140
49	+10 to +19	+16	68	55 to 85	109	91 to 140
38	+20 to +29	+25	70	57 to 98	115	95 to 163
13	+30 to +39	+35	79	66 to 104	120	97 to 149
11	+40 to +49	+45	84	72 to 97	129	114 to 140
6	+50 to +59	+54	90	60 to 120	138	100 to 171
7	+60 to +69	+63	95	80 to 108	142	114 to 170
4	+70 to +79	+74	102	84 to 118	150	120 to 169
1	+80 to +89	+81	94	94	134	134

* The basal pulse rate in percentage is calculated from the actual pulse rate, by assuming a rate of 60 as 100 per cent for men and 70 as 100 per cent for women.

elevated. When fever exists, the basal metabolic rate is undoubtedly elevated to a greater height than it would be if the patient were afebrile. A correction for this factor may be accomplished by the application of DuBois' work [12] wherein he allots an increase of 7.2 per cent for each degree (F.) of fever. Since only a small percentage of the patients under observation were febrile at the time of the metabolism determination, and since the increased body temperature when present was usually slight, this factor has been disregarded, although it is recognized that it introduces a slight error.

BASAL METABOLISM AND PULSE RATE

Minot and Means [13] have investigated the correlation between the basal pulse rate and the basal metabolism in chronic myelogenous and

12. DuBois, E. F.: Basal Metabolism in Health and Disease, ed. 1, Philadelphia and New York, Lea and Febiger, 1924, p. 330.

13. Minot, G. R., and Means, J. H.: The Metabolism-Pulse Ratio in Exophthalmic Goiter and Leukemia, Arch. Int. Med. **33**:576 (May) 1924.

chronic lymphatic leukemia, and they have found a high degree of parallelism between the two. From their data it appears that there is an average increase in the basal pulse rate of 17 per cent for each 10 per cent rise in the basal metabolism. They note that the rise in the pulse rate in leukemia is similar to that demonstrated by Sturgis and Tompkins [14] for exophthalmic goiter. In our patients, who had chronic myelogenous leukemia only, there was an average increase in the basal pulse rate of about 7 per cent for every 10 per cent rise in the basal metabolism. Table 3 shows the relationship between the pulse rate a minute and the basal metabolism in 242 determinations. The increase in the average pulse rate for each 10 per cent rise in the basal metabolic rate is fairly uniform. The range of variation in the pulse rate and in the basal pulse rate is shown in separate columns. While there is

TABLE 4.—*The Relation of the Basal Metabolic Rate to the Average Red Blood Cell Count in Chronic Myelogenous Leukemia*

Number of Determinations	Range of Basal Metabolism	Average Basal Metabolism	Average Red Count (Millions) per Cc.	Range of Red Count (Millions) per Cc.
3	−20 to −11	−14	3.88	3.79 to 4.10
45	−10 to − 1	− 5	4.08	3.16 to 5.37
55	0 to + 9	+ 5	3.68	1.88 to 5.28
32	+10 to +19	+14	3.12	1.86 to 4.55
26	+20 to +29	+24	3.31	2.44 to 4.64
5	+30 to +39	+35	3.71	3.57 to 3.90
5	+40 to +49	+44	3.59	2.02 to 4.43
3	+50 to +59	+54	3.09	2.34 to 3.57
6	+60 to +69	+63	2.11	1.54 to 3.51
3	+70 to +79	+72	2.38	1.57 to 3.45
1	+80 to +89	+81	2.69	2.69

considerable variation in each patient, the low and high extremes, when plotted in the form of a curve, rise uniformly. The individual variations in patients with a similar degree of elevation of the basal metabolic rate are possibly accounted for by the numerous other factors which influence the pulse rate, such as anemia and mental excitement during the tests.

BASAL METABOLISM AND ANEMIA

Patients who have myelogenous leukemia frequently have anemia, although in the early stages of the disease this may be absent or slight, while in the later stages it is usually severe. From the figures shown in table 4, it appears that the severity of the anemia bears no definite relation to the degree of elevation of the basal metabolic rate. When the metabolism is greater than plus 60, however, the anemia is usually more marked than when the metabolism is lower. This observation may be explained on the basis that the basal metabolism is high in the last

14. Sturgis, C. C., and Tompkins, E.: The Correlation of the Basal Metabolism and Pulse Rate in Patients with Hyperthyroidism, Arch. Int. Med. **26**:467 (Oct.) 1920.

stages of the disease when the anemia is an associated condition but has no causal relationship to the high metabolism. The lack of uniformity between the basal metabolic rate and the number of red blood cells precludes the possibility that changes in the number of the red blood cells play any important rôle in elevating the basal metabolism.

RELATION OF BASAL METABOLISM TO WHITE CELL COUNT AND IMMATURITY OF WHITE BLOOD CELLS

Previous investigators have emphasized the relationship between the number of white blood cells in the peripheral blood and their degree of immaturity to the level of the basal metabolism in chronic myelogenous leukemia. Our own observations are presented in tables 5, 6, 7 and 8.

While it is difficult to draw accurate conclusions from general averages, it appears from the figures presented in table 5 that there is

TABLE 5.—*The Average White Blood Cell Count in Relation to the Basal Metabolism in Chronic Myelogenous Leukemia*

Number of Estimations	Range of Basal Metabolic Rate	Average Basal Metabolic Rate	Average White Count (Thousands) per Cc.	Range of White Count (Thousands) per Cc.
3	−20 to −11	−14	13.3	12.2 to 14.9
48	−10 to − 1	− 5	35.3	7.9 to 133.6
63	0 to + 9	+ 5	72.2	9.2 to 220.5
38	+10 to +19	+15	118.1	6.8 to 360.0
34	+20 to +29	+24	189.0	24.2 to 385.0
8	+30 to +39	+35	220.1	18.0 to 704.0
7	+40 to +49	+45	111.4	13.0 to 215.0
4	+50 to +59	+54	290.8	213.2 to 342.4
5	+60 to +69	+63	142.2	12.2 to 336.8
3	+70 to +79	+72	129.8	43.4 to 241.0
1	+80 to +89	+81	342.0	342.0

a gradual increase in the average white cell count as the metabolism rises until the basal metabolic rate reaches plus 40. Above this point the average numbers of white cells for each ten point increase in the basal metabolic rate is irregular and shows no constant tendency to increase. It seems possible, therefore, that there may be other factors of importance which influence the rate of oxygen consumption.

A consideration of the numerical level of the white blood cell count and the basal metabolism shows, as Gunderson [6] has observed, that there is not always a close correlation between the two. As shown in table 5, for each 10 per cent range of the basal metabolism either extremely low or greatly elevated white counts may be encountered, although usually high counts occurred with a high metabolism and low counts with a low metabolism.

In table 6 the percentage of the immature cells is compared with the basal metabolic rate. All white blood cells of the myelocyte series were considered to be immature. As a rule, a high basal metabolic rate appears to be associated with the occurrence of a high percentage of

immature cells in the blood stream. This is not, however, an invariable observation, as a study of the variation of the percentage of immature cells within each 10 per cent range in the basal metabolic rate indicates that in some instances a high or low basal metabolic rate may be associated with either a high or a low percentage of immature cells. The actual number of immature cells per cubic millimeter of blood did not show any closer correlation with the basal metabolism than the percentage number (table 7).

TABLE 6.—*The Percentage of Immature Myeloid Cells in Relation to the Basal Metabolism in Chronic Myelogenous Leukemia*

Number of Estimations	Range of Basal Metabolic Rate	Average Basal Metabolic Rate	Average of Immature Cells, Percentage	Range of Immature Cells, Percentage
3	−20 to −11	−14	12.3	10.5 to 14.0
45	−10 to − 1	− 5	20.1	10.5 to 37.0
53	0 to + 9	+ 5	19.1	2.0 to 32.0
33	+10 to +19	+15	21.7	6.0 to 37.0
32	+20 to +29	+24	25.1	5.0 to 43.0
7	+30 to +39	+34	25.9	3.0 to 48.5
6	+40 to +49	+45	20.6	11.0 to 29.0
3	+50 to +59	+53	33.0	29.0 to 36.0
5	+60 to +69	+63	45.0	15.0 to 67.0
3	+70 to +79	+72	41.0	30.0 to 56.0
1	+80 to +89	+81	86.0	86.0

TABLE 7.—*The Number of Immature Myeloid Cells per C. mm. in Relation to the Basal Metabolic Rate in Chronic Myelogenous Leukemia*

Number of Estimations	Range of Basal Metabolic Rate	Average Basal Metabolic Rate	Average Number of Immature Cells (Thousands) per C.mm.	Range of Number of Immature Cells (Thousands) per C.mm.
3	−20 to −11	−14	1.64	1.35 to 1.79
45	−10 to − 1	− 5	7.67	1.24 to 37.40
54	0 to + 9	+ 5	14.65	0.11 to 59.54
32	+10 to +19	+15	31.15	1.15 to 114.68
33	+20 to +29	+24	49.26	1.21 to 121.19
8	+30 to +39	+35	129.08	0.87 to 647.68
6	+40 to +49	+45	23.11	2.08 to 54.83
3	+50 to +59	+53	98.54	61.83 to 117.36
3	+60 to +69	+64	73.59	55.69 to 105.52
3	+70 to +79	+72	53.66	13.02 to 89.17
1	+80 to +89	+81	294.12	294.12

Table 8 shows the average percentage of myeloblasts and myelocytes for each 10 per cent range in the basal metabolic rate. This table illustrates that the percentage of myelocytes showed no steady increase compared to that of the basal metabolic rate, while the average increase in the percentage of the most immature forms, the myeloblasts exhibited a rather close relationship. This statement is not without exception, however, as indicated by the variation in the percentage of these immature cells within each 10 per cent range of the basal metabolic rate. It has been assumed that the presence of many young cells, such as myeloblasts or atypical young forms of cells, in the peripheral blood indicates a marked overactivity of the bone marrow which is com-

parable to a rapidly growing malignant tumor. If this assumption is correct, it seems probable that at least a part of the rise in oxygen consumption by leukemic patients may be caused by an increased demand for oxygen by the overactive white blood cell forming tissues.

RELATION OF BASAL METABOLISM TO CONDITION OF PATIENT

Grafe [5] made the most important observation that the severity of the leukemic process and the degree of elevation of the basal metabolism were closely associated with each other. In a review of the clinical histories of the patients studied, this is most apparent. In each patient on whom determinations of the basal metabolic rate were made at frequent intervals during the course of his illness, there appeared to be close agreement between the intensity of the symptoms and the degree of elevation of the metabolism. Clinical improvement after irradiation

TABLE 8.—*The Percentage of Myeloblasts and Myelocytes in Relation to the Basal Metabolism in Chronic Myelogenous Leukemia*

Number of Estimations	Range of Basal Metabolism	Average Basal Metabolism	Myelocytes, Percentage	Range of Myelocytes, Percentage	Myeloblasts, Percentage	Range of Myeloblasts, Percentage
3	−20 to −11	−14	12.0	10.5 to 14.5	0.3	0 to 0.5
45	−10 to − 1	− 5	19.4	10.0 to 35.0	0.5	0 to 3.5
53	0 to + 9	+ 5	18.3	2.0 to 29.0	0.8	0 to 3.5
33	+10 to +19	+15	20.4	6.0 to 35.5	1.3	0 to 7.0
32	+20 to +29	+24	22.6	4.0 to 41.0	2.2	0 to 8.5
7	+30 to +39	+34	22.4	3.0 to 45.0	3.5	0 to 9.0
6	+40 to +49	+45	16.9	4.0 to 25.5	4.7	0 to 25.0
3	+50 to +59	+53	29.7	21.0 to 36.0	3.3	0 to 8.0
5	+60 to +69	+63	32.4	15.0 to 45.5	12.5	0 to 29.0
3	+70 to +79	+72	29.8	26.5 to 33.0	10.8	0 to 22.0
1	+80 to +89	+81	65.0	65.0	21.0	21.0

likewise parallels the fall in the metabolism. Patients who had not recently been irradiated, and who showed the earliest symptoms of a recurrence had a moderately elevated metabolism. Those who were severely ill had a uniformly high metabolism. Those who had few symptoms, despite an elevated white blood cell count, showed a low or slightly elevated basal metabolism. It is important to recognize that, in general, the degree of the elevation of the basal metabolism seemed far more in harmony with the clinical condition of the patient than with either the white blood cell count or the differential blood count. It is not surprising that this is true, as many of the distressing symptoms associated with myelogenous leukemia, such as dyspnea, tachycardia, increased sweating, sensation of increased body warmth, loss of weight and intolerance to heat are dependent on the increased metabolism. In this respect the clinical use of the basal metabolic rate determination in leukemia is of value in the same fashion as it is in hyperthyroidism. It gives an index of the activity of the disease which is often difficult to obtain by any other single method of study.

Three patients, who are mentioned in the foregoing, were considered separately as they showed unusually acute manifestations of the disease. All were young women in whom the disease ran a rapid and acute course. In each case there was pronounced anemia, and the white blood cells were increased only moderately. The percentage of immature myeloid cells, however, was great and most of them were myeloblasts. In one patient the basal metabolic rate was plus 32, the pulse rate 104 a minute, the red blood cell count 1,400,000 a cubic millimeter and the white blood cell count 53,600 a cubic millimeter, of which 92 per cent were atypical myeloblasts. In the second patient the basal metabolic rate was plus 39, and the pulse rate was 104 a minute. Blood counts were not made on the day on which the metabolism test was performed, but previous counts showed 97 per cent of the white cells to be immature forms. In the third patient the basal metabolic rate on three occasions was plus 60, plus 51 and plus 40; the pulse rate averaged about 104 a minute. Blood counts were obtained at the time of the last two metabolism determinations. At these times the red blood cell count was 2,000,000 and 1,400,000 a cubic millimeter; the white blood cell counts were 60,200 and 28,400 a cubic millimeter, and the differential counts showed that about 90 per cent of the white blood cells were myeloblasts, and that 1 per cent were myelocytes. In these patients the degree of elevation of the basal metabolic rate seemed to be associated more with the greater degree of immaturity than with the number of white cells in the blood.

INTENSIVE DAILY STUDY OF BASAL METABOLISM AND BLOOD PICTURE: REPORT OF CASES

The preceding statistical study has many disadvantages. The most serious of these is that a dynamic physiologic process like the basal metabolism is difficult to understand when expressed in fixed terms, such as the averages of a group of isolated estimations. Likewise a single examination of the blood at any instant may give an inadequate picture of the preceding blood changes which may have been responsible for the level of the metabolism.

In normal persons the basal metabolic rate is fairly constant from day to day. Even in persons having hyperthyroidism and a greatly elevated basal metabolic rate, a similar constancy in the basal metabolism may be seen. In patients with myelogenous leukemia, as will be shown later, there is a tendency to a more marked daily fluctuation as the rate of oxygen consumption rises and falls irregularly, and the extent of the variation from day to day often exceeds 10 per cent of the normal metabolism. A similar daily variation in the blood picture of chronic myelogenous leukemia likewise was noted. White blood cell counts which varied as much as 150,000 a cubic millimeter from one day to the

next were observed, and a daily difference in the white blood cell count of 50,000 cells a cubic millimeter was not unusual. For example, in case 1 the white blood counts on five successive days before treatment were 233,500, 329,500, 177,000, 248,000 and 198,000, respectively, a cubic millimeter. There was also a considerable daily change in the percentage of immature myeloid cells. Moreover, it is not uncommon to observe a difference of a million cells in the red blood count from one day to the next. The fallacy of stressing the relation of the white blood cell count at any given time to the basal metabolic rate is obvious

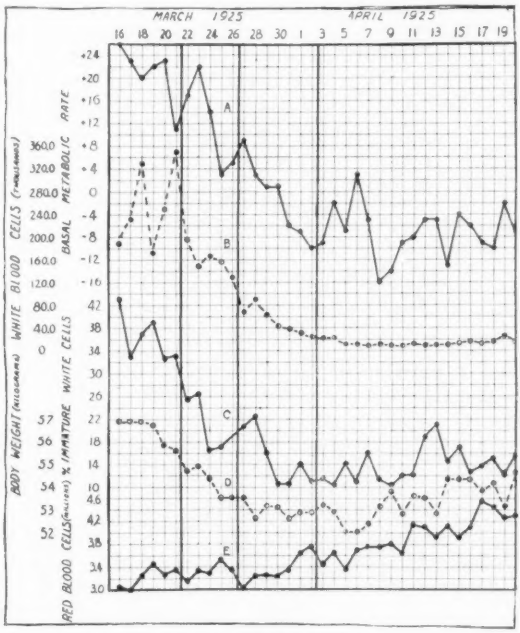

Chart 1 (case 1).—Before, during and after treatment with roentgen rays (first entry). *A*, indicates the basal metabolic rate; *B*, the number of white blood cells per cubic millimeter; *C*, the percentage of immature white cells; *D*, the body weight in kilograms; *E*, the number of red blood cells per cubic millimeter for thirty-six consecutive days in a patient with chronic myelogenous leukemia. The times of irradiation are represented by heavy vertical lines.

unless data concerning both are obtained simultaneously and at frequent intervals. Both are rapidly changing from day to day, rarely in the same direction, as may be seen by charts 1 to 7. Any isolated study of the white blood cells shows the condition of the peripheral blood at a single instant but does not give a clear indication of the labile, dynamic changes occurring in the blood stream and the blood-forming tissues which are probably associated with the fluctuation in

the basal metabolic rate. In order, therefore, to obtain more accurate information bearing on this relationship, daily observations for various periods of time were made on five patients with chronic myelogenous leukemia. These studies include observations before, during and after treatment with short wave length roentgen rays. As the following patients have been studied intensively, a brief summary of the history and physical examination of each is given below:

CASE 1.—B. M., a white man, unmarried, a mechanic, aged 36, had been ill for four years. The most prominent symptoms were loss of strength and weight,

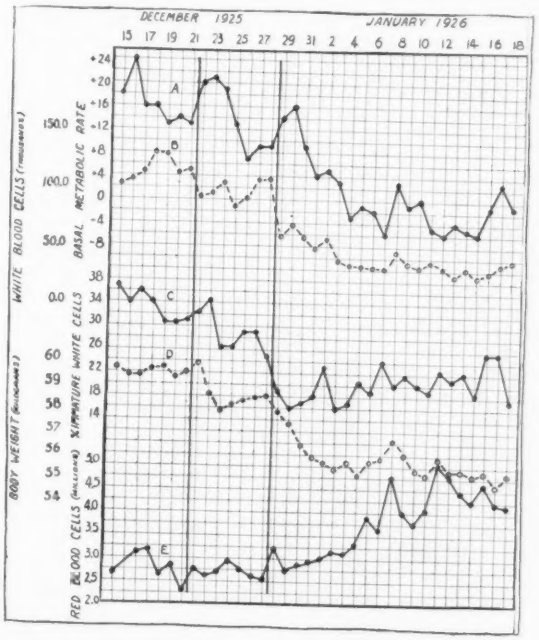

Chart 2 (case 1).—Before, during and after roentgen-ray treatment (second entry). *A*, indicates the basal metabolic rate; *B*, number of white blood cells per cubic millimeter; *C*, percentage of immature white blood cells; *D*, body weight in kilograms; *E*, the number of red blood cells per cubic millimeter for thirty-five consecutive days in a patient with chronic myelogenous leukemia. Treatment by roentgen-ray irradiation is indicated by heavy vertical lines.

recurrent epistaxis and bleeding from hemorrhoids and vague pains in the back. The essential physical observations were pallor of the skin and mucous membranes, profuse sweating, hemorrhoids and a greatly enlarged spleen. He had received no previous treatment. He was studied on three occasions; first, beginning March 15, 1925, for thirty-six days, again for thirty-five days beginning Dec. 14, 1925, and again for thirty-three days beginning March 25, 1926. During the observation periods his temperature was never elevated above normal. The observations made are presented in charts no. 1, 2 and 3.

Case 2.—B. F., a white man, unmarried, an electrician, aged 49, had been ill for four years. Oustanding symptoms were weakness, dyspnea, palpitation, increased sweating, increased appetite, loss of weight and inconstant vague pain in the upper right quadrant of the abdomen. On several occasions there was hemorrhage into the gastro-intestinal tract. The important physical observations were a greatly enlarged spleen, a barely palpable liver and a hot, moist, pale skin. During the period of observation his temperature was never above normal. He had received roentgen-ray therapy five months before he came under our observation. His basal metabolic rate on previous occasions had been distinctly elevated. He was studied on two occasions, and the observations are presented in chart 4.

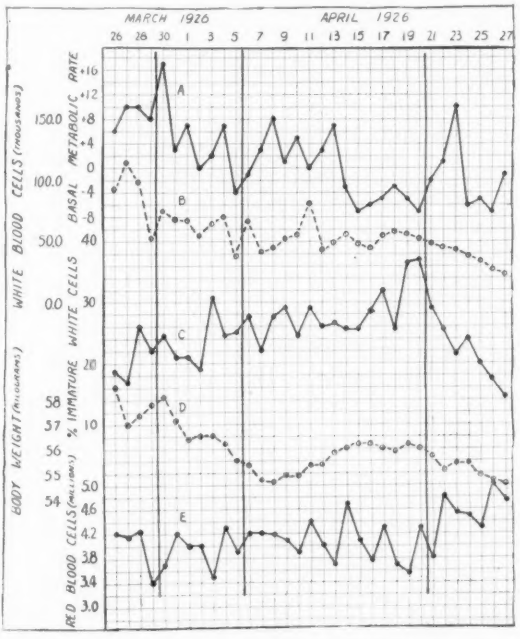

Chart 3 (case 1).—*A*, indicates the basal metabolic rate; *B*, number of white blood cells per cubic millimeter; *C*, percentage of immature white blood cells; *D*, body weight in kilograms; *E*, the number of red blood cells per cubic millimeter in a patient with chronic myelogenous leukemia (third entry) on thirty-three consecutive days, before, during and after treatment with the roentgen ray. Treatment by irradiation with the roentgen ray is indicated by heavy vertical lines.

Case 3.—H. S., a mechanic, aged 35, had been ill for about eight months. The principal symptoms were weakness, diarrhea, loss of weight, intra-ocular and gastro-intestinal hemorrhage and undue bleeding following tonsillectomy at the onset of his illness. Physical observations were a moderately enlarged spleen and liver and pallor of the skin and mucous membranes. He had never been treated with roentgen rays or radon. He was afebrile during the period of observation, which lasted for fifteen days, beginning April 22, 1926. The observations on this patient are plotted in chart 5.

CASE 4.—M. K., a white, widowed housewife, aged 52, had been ill for three years. The important symptoms were weakness, loss of weight, dyspnea, nausea, palpitation, cough and subcutaneous hemorrhages. She exhibited marked symptoms of increased metabolism such as increased feeling of warmth, excessive perspiration and intolerance to heat. Physical examination showed a sick, emaciated woman with a pale, hot, moist skin, an enlarged heart, spleen and liver. She had an elevated body temperature rising as high as 102 F. at intervals during the period of observation, which lasted for six days. Necropsy showed the observations characteristic of myelogenous leukemia. Prior to this period of observation she had received numerous treatments with the roentgen rays, the last treatment having been given about two months before death. The observations made on this patient are given in chart 6.

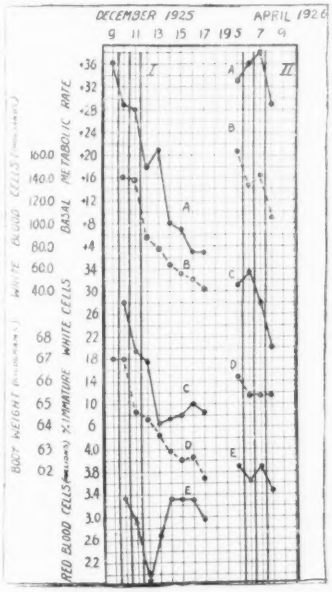

Chart 4

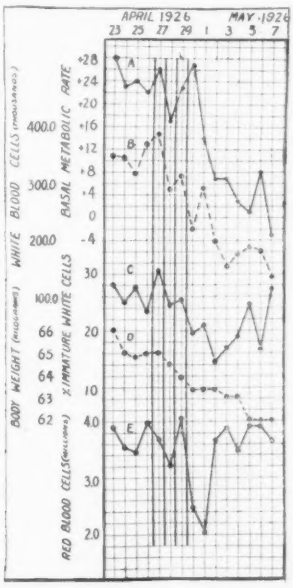

Chart 5

Chart 4 (case 2).—*A*, indicates the basal metabolic rate; *B*, number of white blood cells per cubic millimeter; *C*, percentage of immature white blood cells; *D*, body weight in kilograms; *E*, the number of red blood cells per cubic millimeter in a patient with chronic myelogenous leukemia. The patient was studied on two occasions (1, first entry for nine days and 2, second entry for four days). The roentgen-ray treatment is indicated by heavy vertical lines.

Chart 5 (case 3).—*A*, indicates the basal metabolic rate; *B*, number of white blood cells per cubic millimeter; *C*, percentage of immature white blood cells; *D*, body weight; *E*, the number of red blood cells per cubic millimeter in a patient with chronic myelogenous leukemia on fifteen consecutive days before, during and after treatment with roentgen rays. Treatment by irradiation is indicated by the heavy vertical lines.

CASE 5.—M. M., a white housewife, aged 47, had been ill for two years. The most important symptoms were loss of weight and strength, diarrhea, excessive body warmth and increased tolerance to cold weather. The essential physical

observations were a pale, hot, dry skin, slightly enlarged heart and greatly enlarged spleen. She had been treated previously with the roentgen ray, the last treatment having been given five months before this admission. She had an elevated body temperature throughout the period of observation which lasted for four days and terminated with death. Observations on this patient are given in chart 7.

The first patient was studied on three occasions. The periods lasted for thirty-six, thirty-five and thirty-three days, respectively. The second patient was studied on two occasions; once for nine days and again for four days. The third patient was studied on one occasion for fifteen days. The fourth and fifth patients, both in the terminal stages of the disease, were each studied for six and four days, respectively. In the two latter cases death terminated the observations. In these studies daily observations were made on the basal metabolic rate, red

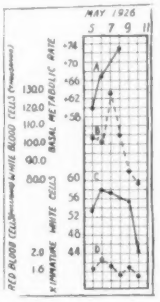

Chart 6

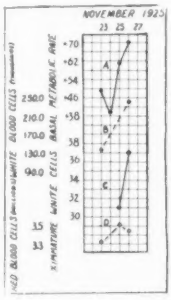

Chart 7

Chart 6 (case 5).—*A*, indicates the basal metabolic rate; *B*, the number of white blood cells per cubic millimeter; *C*, the percentage of immature white blood cells; *D*, the number of red blood cells per cubic millimeter in a patient with chronic myelogenous leukemia in the terminal stages. The patient died after six days of observation.

Chart 7 (case 4).—*A*, indicates the basal metabolic rate; *B*, the number of white blood cells per cubic millimeter; *C*, the percentage of immature white blood cells; *D*, the number of red blood cells per cubic millimeter in a patient with chronic myelogenous leukemia in the terminal stages. The patient died after four days of observation.

cell count, white cell count and hemoglobin content of the blood. Each day a differential white blood count was made on at least 200 cells. All observations were made under similar basal conditions and with standardized instruments.

Certain interesting facts, recorded below, may be observed from the charts (1 to 5). It is apparent that while the total white blood cell count and the percentage of immature myeloid cells rise and fall in a general way with the basal metabolic rate, the minor fluctuations of the basal metabolism from day to day do not correspond to the variations

in the white cell count. The red blood cell count and the percentage of hemoglobin in the blood appear to rise as the metabolism falls.

When roentgen-ray treatments are given at intervals of time sufficiently long to determine the effect of each dose, as in one patient (case 1, charts 1, 2 and 3), each exposure appears to have had a definite effect both on the cellular composition of the blood and on the basal metabolic rate. The basal metabolism after each irradiation showed an abrupt and temporary rise, lasting from one to three days, after which it fell, usually to a level lower than that before treatment. Following this transient rise there was an interval when the metabolism remained about stationary, after which it again decreased. It is significant that the initial transitory rise in the basal metabolic rate was accompanied by a fall in the total white blood cell count and in the percentage of immature white blood cells. It is also observed, on close scrutiny of charts 1, 2 and 3, that the fluctuations from day to day in the total white and differential cell count on the one hand and in the basal metabolic rate on the other appear in most cases to vary in opposite directions. As the white cell count and the percentage of immature white cells increased, the basal metabolic rate decreased, and as the former decreased, the latter increased. If a longer period of time is considered, such as a week or ten days, it will be seen that there is a general tendency of the curves of these factors to parallel each other. The failure of the number of white blood cells or the percentage of immature ones to parallel constantly the basal metabolism has been referred to before. It suggests that another factor or other factors play a rôle in producing the increased metabolism. The most logical cause to be considered is the one originally suggested by Grafe,[5] who thought that an overactivity of the myeloid tissues may increase the metabolism without evidence of this being clearly shown by the peripheral blood picture, as occurs in aleukemic phases of different kinds of leukemia. Therefore, one might attribute logically an increased metabolism to an overactivity of the blood-forming tissues, which for some unknown reason does not cause a true leukemic picture in the peripheral blood. Another partial cause of the elevated metabolism may be an abnormal increase in destruction of the white blood cells, which is discussed below.

In cases 2 and 3 (charts 4 and 5) the observations were somewhat different from those in case 1 (charts 1, 2 and 3). The former were treated with short wave length roentgen-ray exposures, which were given daily for from four to six days, while the latter were given single treatments at intervals of at least several days. In the more intensive method of treatment the effects of several exposures were superimposed one on the other and, therefore, the character of the change in the basal metabolic rate and the cellular composition of the

blood is different. The typical transitory rise in the basal metabolic rate following treatment in these patients is absent. Instead, it decreased, remained stationary or increased slightly during the several days of treatment. Following the course of treatment there was a fall in the metabolism, the white blood cell count and the percentage of immature white cells.

The results in case 4 (chart 6) and case 5 (chart 7) illustrate the relationship between the basal metabolic rate and the blood observations in the terminal stages of the disease. Fever, which was present in both patients, slightly modifies the results. The metabolism rose rapidly in both patients as death approached. This rise was not accompanied in case 4 by an increase in the number of white blood cells or in the percentage of immature white cells, although the latter were numerous. In this patient there was an actual decrease in the number of white blood cells as death approached. More data could not be obtained concerning these two patients on account of their serious condition. The observations in both patients indicate that the basal metabolism was a more accurate index of their condition than any other single criterion. In case 5 the patient appeared to be in a fair condition, and there was no unusual elevation of the number of white blood cells or the percentage of immature cells. The basal metabolism was high (plus 49) on the initial determination and reached plus 70 three days later. The observations were terminated by the patient's rather unexpected death. In case 4, the patient likewise had a high rate of metabolism and a relatively low white blood cell count. She died after an observation period of six days. In addition to the high rate of metabolism the seriousness of her condition was indicated by her appearance and by the high percentage of immature cells. These studies, during the terminal stage of the disease, indicate as Means and Lennox [15] have emphasized, that a persistently high rate of basal metabolism in patients with myelogenous leukemia suggests a poor prognosis, although the observations on the blood may not in themselves be extremely unusual. From our experience it also appears to be true that patients who have a low basal metabolic rate, in association with a high white cell count, are probably in much better condition than the blood count indicates. This point is illustrated by patients (cases 1 and 3) who had a high white blood cell count with a large percentage of immature cells in association with a moderate elevation of the basal metabolism. They were not so seriously ill as the blood picture alone would suggest, and the response to treatment in both patients was prompt.

15. Means, J. H., and Lennox, W. G.: A Study of the Basal and Nitrogenous Metabolism in a Case of Acute Leukemia During Roentgen-Ray Treatment, Arch. Int. Med. **32**:705 (Nov.) 1923.

COMMENT

The underlying causes of the increased basal metabolism in chronic myelogenous leukemia are not entirely clear. The commonly accepted theory is the one originally advanced by Grafe,[5] in which he suggests that the elevated metabolism is due to three causes: (*1*) an increased consumption of oxygen by the leukemic blood, (*2*) the use of oxygen by the leukemic cells elsewhere in the body than in the peripheral blood, (*3*) the increased demand for oxygen by the overactive tissues which are responsible for the production of the large numbers of white blood cells. It seems a reasonable deduction to conclude that the first factor is of some importance, and it is supported by the experiments of Grafe [5] on leukemic blood in vitro. This investigator calculates, however, that in patients with lymphatic leukemia in whom there is a tremendous increase in lymphocytes, the circulating blood accounts for only about 10 per cent of the total oxygen consumption by the patient. Moreover, in patients with a total white blood cell count in the vicinity of 200,000 per cubic millimeter, the elevation of the oxygen consumption by the blood alone is trivial, as he estimates that it accounts for only 3 per cent of the total amount of oxygen which is consumed. The use of oxygen by white blood cells stored in various tissues of the body and by the overactive hematopoietic tissues are factors which are difficult to evaluate. It appears reasonable to suppose that they do play a rôle in the elevation of the metabolism, but it is impossible to estimate accurately their importance.

As it does not seem that the suggestions of Grafe,[5] are adequate in accounting for the entire increase in metabolism, we have considered other factors that might be important. The only additional one that seems worthy to evaluate is the character and the rate of destruction of the white blood cells. Very little is known about this, but it seems possible that it may have some relation to the level of metabolism. This is suggested because directly following irradiation, large numbers of white blood cells were destroyed and the rate of metabolism increased, while the total white cell count and the percentage of immature white blood cells diminished. The rise was not caused by fever, as the patients showing this phenomenon most clearly were afebrile throughout the period of observation. It is also interesting to recall that there was usually no parallelism between the minor fluctuations of the basal metabolism from day to day and the total white cell count or the percentage of immature white cells. On the contrary, there was more of a tendency to an inverse relationship. The possibility of a relationship between the metabolism and destruction of the white blood cells is offered as a suggestion, which has not been proved. It is probable that other unknown factors likewise are of importance in this connection.

SUMMARY

1. The basal metabolic rate in untreated patients with myelogenous leukemia is elevated with few exceptions above the normal limits, although in some instances the increase is slight. In general, the degree of elevation depends on the severity of the leukemic process and is of value in indicating the prognosis.

2. Irradiation of the patients with roentgen rays usually produces a transitory rise in the basal metabolic rate for not more than three days. In patients who are benefited by treatment this rise is followed by a rapid fall to normal limits, where it usually remains for from three to six months and in some instances even longer. Patients in the terminal stages of the disease, however, continue to have an elevated metabolism despite treatment.

3. There is a direct correlation between the pulse rate and the degree of elevation of the metabolism.

4. There appears to be no fixed relationship between the basal metabolic rate and the degree of anemia.

5. The basal metabolism is frequently high with high white blood counts and low with low white blood counts.

6. The basal metabolism is usually high when the percentage of the young myeloid cells is great, and especially when their degree of immaturity is marked.

7. Intensive daily studies showed that there appeared to be an inverse relationship between the minor fluctuations of the basal metabolic rate from day to day, on one hand, and fluctuations of the number of white blood cells and the percentage of immature forms, on the other hand. The general level of the basal metabolic rate, however, depended on the degree of elevation of the total white blood cell count and of the percentage of immature myeloid cells.

8. In addition to the commonly assumed causes of the elevation of the metabolism in myelogenous leukemia, it is suggested that the increased rate of destruction of the white blood cells may be a factor of importance.

BILIARY, PANCREATIC AND DUODENAL STUDIES

I. THE HYDROGEN ION CONCENTRATION OF SUCCESSIVE PORTIONS OF DUODENAL CONTENTS FOLLOWING STIMULATIONS WITH MAGNESIUM SULPHATE

LAY MARTIN, M.D.

BALTIMORE

Among the latest and most important observations on the hydrogen ion concentration of the human duodenum are those made by Hume, Dennis, Silverman and Irwin.[1] Their subject was a colored man who had suffered a gunshot wound, the cause of which was unknown, and from which a duodenal fistula resulted. Their conclusions were that, "Electrometric determination of the hydrogen ion concentration of the human duodenum made by the insertion of an electrode through a fistulous opening gave a maximum p_H 8.23 and a minimum p_H 5.91; while the average calculated from 182 readings was p_H 7.02. No definite difference in reaction was noted in observations made after the ingestion of meals consisting of fat, carbohydrate or protein."

McClure, Montague and Campbell,[2] having obtained their material in a more customary manner, i. e., via a duodenal tube, secured results comparable to those of the aforementioned writers. Their material was obtained from normal men. They used a Leed-Northrup potentiometer in their estimations. The observations were made, either as the patients were fasting, after they had drunk tap water, or after various single or mixed foodstuffs had been ingested. With the exception of three of their estimations, the p_H values lie between 6 and 8.102. Of the three exceptions one of p_H 3.172 was between 5.101 and 7.013; the p_H 5.101 was between 8.102 and 3.172. These two low readings followed the ingestion by mouth, of edestin. However, in two other instances edestin was given and followed by a relatively high p_H, certainly comparable to those obtained after the ingestion of other foodstuffs. The other reading p_H 5.961 followed the oral administration of olive oil, but was obtained before the "onset" of intestinal digestion. Their conclusions are that: 1. The p_H of duodenal contents varied after the ingestion of various food substances. The duodenal contents were acid

1. Hume, H. V.; Denis, W.; Silverman, D. M., and Irwin, E. L.: Hydrogen Ion Concentration in the Human Duodenum, J. Biol. Chem. **60**:633 (July) 1924.

2. McClure, C. W.; Montague, A. C., and Campbell, L. L.: The p_H and Buffer Values of Duodenal Contents Derived from Normal Men, Arch. Int. Med. **33**:525 (April) 1924.

after the ingestion of protein and mixtures of food substances, and alkaline after the ingestion of fat and carbohydrate food substances.

2. No relation was found between the p_H of the duodenal contents and the stimulation of the flow of bile and pancreatic juice. The p_H obtained either while the patient was fasting or after the ingestion of tap water was always alkaline.

McClendon,[3] in 1915, using his own potentiometer method, ascertained that, "The adult duodenal contents are slightly alkaline. The hydrogen ion concentration is about 0.00000002!" He also made this interesting statement, "The infant's duodenum is more acid than the average acidity of the stomach. Pepsin is always present and peptic digestion must take place." He also said, "The less acid samples have a greater admixture of bile. The bile is not sufficient to make the duodenal contents alkaline." As I shall show in a subsequent paper, what he is proba-

TABLE 1.—*Variations of pH After the Ingestion of Various Foods*

Meal	Food Taken	p_H
Breakfast	Hot cakes, toast, coffee	3.80
	Bacon, rice, coffee	3.20
	Corn flakes and cream, eggs, toast	6.98
Luncheon	Beef, potatoes, tomatoes, pie	4.60
	Pork, potatoes, eggplant, cake	5.00
Breakfast	Hot cakes, pineapple sauce, coffee	7.21
	Toast, raspberry sauce	7.40
	Ham, eggs, toast, coffee	7.54
Luncheon	Beef, potatoes, corn, ice cream	7.00
	Beef, potatoes, bread, melon, iced tea	7.82
	Potatoes, carrots, pie, cake, ice cream, milk	7.60
	Beef, onions, potatoes, pie, cake, followed by bismuth	7.54

bly dealing with here is twofold; a larger flow of bile, and more important, a lessened amount of hydrochloric acid coming through the pylorus. Later in 1920, while working with F. J. Myers,[4] who obligingly swallowed the tube, he found that, "The reaction of the duodenum between 3 and 4 hours after meals was usually found to fluctuate around the neutral point, but the extreme range on the acid side was greater than on the alkaline side, possibly due to the spurting of gastric contents into the duodenum." Table 1 shows the results of McClendon's experiment.

This work well agrees with that done by Long and Fenger [5] in 1917. Using a Rehfuss tube, they found that the p_H values varied from 2.27 to

3. McClendon: Acidity Curves in the Stomachs and Duodenums of Adults and Infants, Plotted with the aid of Improved Methods for Measuring Hydrogen Ion Concentration, Am. J. Physiol. **38**:191, 1915.

4. Myers, F. J., and McClendon, J. F.: Note on the Hydrogen Ion Concentration of the Human Duodenum, J. Biol. Chem. **41**:187 (Feb.) 1920.

5. Long and Fenger: On the Normal Reaction of the Intestinal Tract, J. Am. Chem. Soc. **39**:1278, 1917.

7.81. Their estimations were made after the ingestion of regular meals. On the four subjects observed, the electrometric determinations were made at varying intervals after regular meals. The position of the bulb was controlled by the roentgen ray and varied in position from just beyond the pylorus to the proximal portions of the jejunum. With the tube in any position one is liable to obtain readings on each side of neutrality; their determinations were usually on the acid side. Long and Fenger feel that the passage of gastric chyme has a great deal to do with the p_H of the duodenal secretions, but that perhaps further down one may have to depend on other agencies for the lowered p_H.

In 1922, Okada and Arai,[6] working in Tokyo, reported observations made on fourteen hospital patients. They used the electrometric method, and after removing the duodenal contents at variable times, from one-half to six hours after meals, they found that the p_H varied from 4.8 to 7.97. The lowest readings were obtained in a patient with tabes dorsalis. All the rest were well above 6. The time of removal seems to have no definite effect on the hydrogen ion concentration.

Recently I have had the opportunity to study the hydrogen ion concentration of forty-one patients. The duodenal tube was swallowed in the approved method, and its position in the duodenum was always determined by fluoroscopic examination. At times a free flow of duodenal drainage was instituted by the mere presence of the tube in place. (I am not considering the flow of regurgitant bile sometimes obtained from the stomach.) Observations were made on this at times and were found not to vary from those which my co-workers and I obtained after our routine pancreatic biliary stimulant—40 cc. of 33 per cent magnesium sulphate. This solution produces a free flow of both secretions. The former is recognized by its ferment activity,[7] and the latter by its color. The return flow was divided into separate tubes according to the color concentrations of the bile. It usually fell into the commonly recognized A, B and C fractions. The hydrogen ion concentration of each was estimated and will be reported with other observations that may be of interest or of pertinence.

METHOD

The determinations were made by the colorimetric method. The standards used were those designed by Clark and Lubs[8] and were exactly as recorded in Clark's book.[9] I am certain that the standards are accurate, as many checks were made on them.

Two complete sets were made; one was preserved with triple distilled cresol and the other was autoclaved for twenty minutes at 115 C. So far as can be

6. Okada, S., and Arai, M.: Hydrogen Ion Concentration of Intestinal Contents, J. Biol. Chem. **51**:135 (March) 1922.

7. To be published.

8. Clark, W. M., and Lubs: Hydrogen Electrode Potentials of Phthalate, Phosphate and Borate Buffer Mixtures, J. Biol. Chem. **25**:479 (July) 1916.

9. Clark, W. M.: The Determination of Hydrogen Ions, Baltimore, Williams & Wilkins Co., 1925, p. 81.

determined, both methods act equally well, and, provided that both sets are kept in the dark, they retain their color for considerable time, certainly for a year. Methyl red fades rapidly, and this set of standards must constantly be renewed.

Our method of reading is shown in the diagram.

The box is that ordinarily used for the comparison of color standard sets. The indicator, in amounts equal to that contained in an equal volume of colored standard, is added to the thoroughly centrifugalized duodenal contents. Previous to this, small portions must be tested to determine which indicator will give the best reaction.

The indicators which my co-workers and I used are thymol sulphonphthalein, tetrabrom phenol sulphonphthalein, orthocarboxybenzine azod; methyl aniline, dibromothymol sulphonphthalein, orthocresol sulphonphthalein and orthocresol phthalein.

For the preparation of the stock solutions 100 mg. are ground in an agate mortar with the required amount of twentieth normal sodium hydroxide.[9] When the solution is complete, it should be diluted with water to 25 cc. In making up the methyl red, we dissolved 100 mg. in 100 cc. alcohol and diluted this to 100 cc.

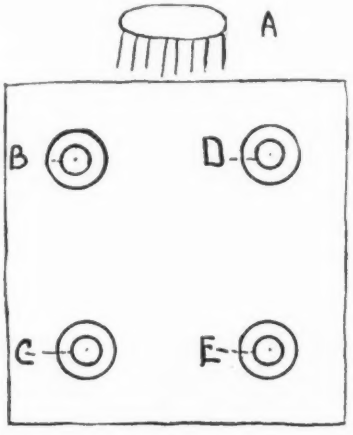

Diagram of method used in reading. *A*, indicates light—substage lantern without blue glass; *B*, distilled water; *C*, unkown plus indicator; *D*, colored standard; *E*, unknown.

All of our tubes were measured for us by Hynson, Westcott & Dunning; the tubes to contain the unknown substance were also of the same standard size and thickness of glass.

A glance at the figures in table 2 will show how relatively rare is a p_H above neutrality. In any series in which such a reading is found there is always one which is acid. This has also been found in two cases of achylia. In another all the readings showed acid.

The only figures in the literature so far reported that can be taken as representing those to be found in the actual fasting state are those of Hume and his associates. Even these are to be slightly questioned as they may be influenced by the opening of the fistula acting as a foreign stimulant.

It is taken for granted that there is but little flow of bile or pancreatic juice during fasting periods. However, frequently there is a free flow of bile following the passage of a duodenal tube. Is this brought about by the action of the bulb on the walls of the duodenum, or by the gastric secretion or débris that must of course follow the passage of the tube through the pylorus?

TABLE 2.—*The p_H in A., B. and C. Bile in Various Diseases*

Diagnosis	p_H in Bile		
	A	B	C
Chronic biliary disease	5.8	5.6	4.8
Angioneurotic edema	4.4	No B	2.2
Chronic arthritis	5.4	4.4	1.2
Chronic arthritis (achylia)	8.0	5.8	8.6
Gallstones	8.4	5.6	2.8
Chronic biliary disease	4.6	6.7	1.3
Migraine	7.0	No B	1.0
Psychoneurosis	5.0	4.4	4.6
Psychoneurosis	4.6	4.6	1.3
Psychoneurosis	5.4	5.8	4.6
Psychoneurosis	5.4	2.0	5.0
Chronic biliary disease	5.8	No B	2.8
Gallstones	6.4	6.2	1.8
Gallstones	6.0	5.8	3.8
Gallstones	4.6	6.2	5.3
Chronic biliary disease	6.0	6.2	5.6
Colitis	6.8	4.6	5.8
Banti's disease	4.0	4.0	2.4
	0.0	7.0	2.4
Chronic biliary disease	8.2	6.8	6.4
Chronic biliary disease	3.8	6.4	1.4
Gallstones	6.6	4.4	3.6
Chronic biliary infection	8.8	6.9	1.8
Arthritis	6.8	7.0	6.4
Choledodrolithiasis	1.6	No B	1.6
Diabetes	3.1	4.4	1.8
Arthritis	5.4	1.8	8.2
Chronic biliary disease	4.0	4.0	1.2
Chronic biliary disease	2.4	4.2	1.2
Chronic biliary disease	7.2	4.2	2.2
Chronic biliary disease	4.2	3.8	1.8
Chronic biliary disease	8.4	6.8	8.2
Migraine	4.8	4.2	3.6
Gallstones	Not done	4.7	1.9
Gumma liver	None	4.2	6.4
Chronic biliary disease	None	6.5	1.2
Gallstones	3.9	4.4	3.9
Psychoneurosis	4.6	6.2	5.3
Chronic biliary disease	6.2	6.0	6.2
Chronic biliary disease	2.4	3.9	None
Psychoneurosis	6.4	4.5	3.7

As noted before, the p_H of this unstimulated flow is comparable to the first secretion to be obtained after magnesium sulphate stimulation. This is the clear, so-called A fraction of which the p_H varies from 2.4 to 8.8, the great majority of the readings, 61 per cent, following between p_H 4.2 and p_H 6.8. Of forty-one determinations, only 20 per cent were alkaline. The hydrogen ion concentration in B bile is approximately the same, with a negligible quantity of alkaline. In the last portion, 61 per cent are below p_H 4. Twenty-nine per cent are between p_H 4 and p_H 6.4. The remainder are alkaline; truly a small representation. In these fractions with such low hydrogen ion values, the presence of free and combined acids was practically always demonstrated by the use of B amidozolethylamin and phenolphthalein.

COMMENT

As noted before, none of the determinations made by my co-workers and myself can be accepted as representing the true fasting state of the duodenum. Our A fraction is probably the same as that which McClure, Montague and Campbell [2] describe as being obtained following the ingestion of food but before the beginning of intestinal digestion. This A fraction is acid in 61 per cent of the cases and usually contains pancreatic ferments, when such are to be obtained in other fractions.

If there is free acid in the stomach, the hydrogen ion concentration of the duodenum obtained through a duodenal tube after stimulations of magnesium sulphate tends in the majority of cases to become more and more acid. This can mean but one thing: Gastric acids are being projected into the duodenum. As this acidity continues, the pancreatic activity and the amount of bile pigments decrease in strength and amount. This is probably due to the fact that the stimulant acts for only a short time and not that the secretions are prohibited by the presence of acid; on the other hand, it may be a pure dilution phenomenon.

There are periods, however, when the pure biliary secretion with a rather high pancreatic activity and a high p_H will alternate with duodenal content of low p_H. Evidently the gastric secretion is coming through in spurts and the undiluted duodenal contents have a p_H bordering on neutrality or just above. From these observations, I feel that the normal duodenal contents per se are practically always nearly neutral, but that any stimulant to the gastric secretion will shortly tend to lower it.

The range of results is found to agree with those of Myer and McClendon,[4] Long and Fenger [5] and Okada and Arai.[6]

CONCLUSIONS

1. After an instillation of magnesium sulphate through a tube directly into the duodenum, the resultant secretion is usually slightly acid. Usually the duodenal contents tend to become more acid the longer the tube is left there. There may be periods of neutrality or even alkalinity.

2. The normal duodenal contents are nearly neutral, but under any digestive procedure they are likely to become acid, and the lowness of the p_H is probably related to the tone of the pylorus sphincter: whether it is to be constantly acid or whether periods of acidity and neutrality will alternate.

3. Even in gastric achylia the duodenal contents are at times acid. Active pancreatic enzymes are found, however, before this chemical change is brought about. They are found in the alkaline fraction.

THE PHYSIOLOGIC EFFECT OF MASSAGE

SECOND CONTRIBUTION *

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Increasing clinical recognition of the value of physiotherapeutic measures of various kinds makes it desirable to determine, if possible, the precise nature of the changes induced by them. Such information is important for the purpose of standardizing procedure and of ascertaining the indications and limits of practice. With the possible exception of the use of heat, massage constitutes the most valuable single form of physiotherapy. While its importance is recognized in many circles, its full value to medicine is still largely unappreciated. For these several reasons, as well as on broad grounds of physiology, a clearer understanding of its effects would have value. In a previous communication,[1] we pointed out that massage differs sharply in its effect from active exercise, with which it would seem to have some analogy. There is considerable evidence that its influence is chiefly, if not solely, through its effect on the circulation, but the real nature of the mechanism concerned has not been fully known.

The physiologic effect of general and abdominal massage has been the subject of considerable work in the past, though in recent years it has attracted little attention from the physiologist or biochemist. The literature up to 1910 has been reviewed by Rosenthal,[2] and his monograph on the scientific basis of massage contains many references to the effect of massage on metabolism. An increased urinary excretion of nitrogen following massage was observed by a number of investigators. The observation of Bendix [3] and also of Voigt [4] may be cited here, since in their experiments, in contrast to some of the earlier work, the food intake of the subjects was carefully controlled. These workers found an increased twenty-four hour nitrogen excretion on days when the sub-

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* From the Laboratory of Clinical Chemistry Presbyterian Hospital.

* The work here reported is part of a study on arthritis in collaboration with Dr. Robert B. Osgood of Boston. The expense of this investigation was defrayed by contributions from several sources, including a number of patients.

1. Pemberton, R.; Cajori, F. A., and Crouter, C. Y.: J. A. M. A. **83**:1761 (Nov. 29) 1924.

2. Rosenthal, Carl: Die Massage und ihre wissenshaftliche Begründung, Berlin, 1910.

3. Bendix, B.: Ztschr. f. klin. Med. **25**:303, 1894.

4. Voight, Otto W.: Inag. Disert. Halle, 1896.

jects received massage, and Voigt also found an increased excretion of phosphorus. Following the massage, several days elapsed before the urinary nitrogen returned to the premassage level. Eccles [5] reports an increased uric acid excretion on days when massage was given. Other evidence of the effect of massage on metabolism is reported by Leber and Stüve,[6] who found a 10 to 15 per cent increase in oxygen consumption and carbon dioxide production, following either general or abdominal massage.

The occurrence of a diuresis following massage has been observed by a number of investigators, Hirschberg,[7] Bendix,[3] Pemberton and their co-workers.[1] Comparable with the excretion of nitrogen, the increase in urinary volume was found to persist for several days.

Most of the work cited in the foregoing was carried out before the advent of the more modern procedures, and in the present studies advantage has been taken of the newer quantitative methods for urinalysis to make a more detailed examination of the urine before and immediately following general and abdominal massage. It was believed that changes, directly referable to the massage, might be found in the rate of excretion of certain urinary constituents. Such changes perhaps would give further information as to the physiologic effects of massage and help to explain the undoubted benefits that result from such measures when applied to many pathologic conditions.

METHODS

All studies were carried out on subjects who had taken a standard breakfast from two to three hours before the collection of urine was begun. This breakfast consisted of one egg, soda crackers, butter and water. Following the breakfast, nothing further was taken until the end of the experiment. Specimens of urine covering an hour to an hour and a half were obtained before the massage, the subject resting during this time. A second specimen of urine was obtained within the half hour following a massage of from thirty-five to sixty minutes' duration, and therefore covered primarily the period of massage. The time was noted carefully, and the hourly excretion of urine calculated for the two periods.

The p_H, specific gravity and titratable acidity were measured at once. The p_H was determined colorimetrically by Felton's [8] spot method. For total acidity, specimens of urine were titrated to a p_H of 7.4 in the manner described by Fiske.[9] Folin's methods [10] were used for the determination of total nitrogen, chlorides and creatinine. The Folin-Benedict-Meyer method [11] was used for the determination of creatinine, modified so that only one-half the quantity of urine and reagents recommended by them was used. Inorganic phosphorus was determined by Brigg's [12] method.

5. Eccles, A. Symons: Practice of Massage, London, 1898.
6. Leber, H., and Stüve: Berl. klin. Wchnschr. **33**:337, 1896.
7. Hirschberg, R.: Bull. général de thérap. **113**:241, 1887.
8. Felton, L. D.: J. Biol. Chem. **46**:299 (April) 1921.
9. Fiske, C. H.: J. Biol. Chem. **49**:163 (Nov.) 1921.
10. Folin, Otto: Laboratory Manual of Biological Chemistry, ed. 3, New York and London, 1922.
11. Benedict, F. G., and Myers, V. C.: Am. J. Physiol. **18**:397, 1907.
12. Briggs, A. P.: J. Biol. Chem. **53**:13 (July) 1922.

TABLE 1.—*Composition of Urine Secreted Before and During a General Massage, Following a Standard Breakfast* *

Case	Date	Volume, Cc. per Hour		Specific Gravity,		p_H		Titratable Acidity Cc. Tenth Normal Acid per Hour		Sodium Chloride, Mg. per Hour		Inorganic Phosphorus, Mg. per Hour		Total Nitrogen, Mg. per Hour		Creatinine, Mg. per Hour		Creatine, Mg. per Hour	
		Before	During	Before	During	Before	During	Before	During	Before	During	Before	During	Before	During	Before	During	Before	During
1	12/15/25	37	21	1.020	.....	6.8	5.8	4.4	5.0	240	150	12	12	279	212	51	41	..	..
2	12/15/25	30	57	1.028	1.027	5.2	5.2	6.2	14.6	440	990	6	27	377	609	46	76	..	..
3	12/21/25	60	60	1.007	1.007	7.0	7.0	2.6	2.1	170	240	15	19	296	329	36	33	..	..
4	12/22/25	21	35	.....	1.021	5.2	5.2	7.6	11.8	190	390	8	20	226	202	41	47	..	..
5	1/21/26	15	25	.....	.....	5.8	5.8	2.5	4.0	28	168	4	12	85	198	33	39	..	..
6	1/25/26	25	54	.....	1.020	5.6	7.0	5.2	3.5	198	492	13	27	165	303	21	34	..	..
7	1/28/26	24	44	1.020	1.021	7.4	7.6	...	...	233	396	8	18	145	247	13	23	..	..
8	1/29/26	80	52	1.018	1.020	5.2	6.4	9.7	4.6	736	440	18	15	498	444	54	53	..	..
9	2/ 5/26	73	99	1.016	1.014	7.6	7.2	...	1.0	458	623	15	26	398	302	45	40	..	..
10	2/ 9/26	33	9	.....	.....	5.0	5.0	9.5	2.1	353	66	17	5	398	90	44	14	..	..
11	2/11/26	136	126	1.015	1.015	5.8	6.4	11.5	7.3	1,990	1,400	25	27	572	437	60	46	7	9
12	2/17/26	35	42	1.016	.....	7.2	7.2	0	0	300	234	8	11	228	238	26	28	.	.
13	2/26/26	60	92	1.011	1.010	5.6	5.4	6.9	8.2	325	512	15	12	315	433	50	71	..	..
14*	3/ 2/26	40	69	1.022	1.018	6.4	7.0	4.7	1.5	474	567	19	20	351	340	41	35	..	..
15*	3/10/26	142	229	1.010	1.007	6.2	6.6	6.7	5.8	832	727	18	26	461	494	75	66	..	..
16*	3/31/26	19	180	1.025	1.010	6.8	6.6	0.3	6.6	179	782	7	32	304	543	26	69	..	..
17*	4/28/26	72	74	1.019	1.020	5.2	5.6	12.5	9.4	729	706	15	13	719	746	72	76	..	..
18*	4/28/26	57	53	1.016	1.014	5.2	5.8	7.6	7.0	337	333	9	14	470	413	42	38	..	..
19*	4/29/26	64	249	1.020	1.011	5.8	7.3	7.4	2.1	686	1,449	17	18	454	753	68	99	..	..
20*	3/24/26	17	31	.....	.....	5.4	6.2	5.7	4.2	319	578	9	6	170	320	47	69	..	..

* Abdominal massage included.

TABLE 2.—*Control Experiments; Composition of Urine Following a Standard Breakfast* *

Case	Date	Volume, Cc. per Hour		Specific Gravity,		p_H		Titratable Acidity Cc. Tenth Normal Acid per Hour		Sodium Chloride, Mg. per Hour		Inorganic Phosphorus, Mg. per Hour		Total Nitrogen, Mg. per Hour		Creatinine, Mg. per Hour		Creatine, Mg. per Hour	
		Before	During	Before	During	Before	During	Before	During	Before	During	Before	During	Before	During	Before	During	Before	During
5	1/22/26	32	35	1.022	1.020	5.8	5.6	5.5	8.2	235	271	13	20	209	222	34	31	..	..
6	1/26/26	..	..	.....	.....	...	7.0	3.3	1.4	473	405	20	16	437	160	40	24	..	..
7	1/27/23	65	45	1.016	1.020	7.4	7.6	...	...	450	393	15	12	331	280	22	20	..	..
3	1/28/26	168	48	1.011	1.015	7.0	6.8	4.4	2.9	437	264	32	16	494	203	55	24	..	..
8	2/ 2/26	47	40	1.022	1.022	5.6	7.6	6.7	...	398	218	8	12	348	407	66	59	..	..
9	2/ 4/26	31	44	1.022	1.025	5.8	7.6	5.5	...	300	400	8	13	258	313	67	48	..	..
10	2/ 8/26	33	25	.....	.....	5.6	5.6	6.6	4.1	201	135	14	10	335	221	37	24	..	..
11	2/ 9/26	54	29	1.020	.....	5.0	5.2	11.9	8.8	502	252	18	17	442	267	64	41	8	8
12	2/12/26	20	34	.....	.....	5.8	7.2	4.2	0.2	342	356	6	7	166	247	31	35	..	..
14	2/26/26	51	107	1.021	1.016	6.4	7.2	4.6	1.7	677	939	23	24	402	482	45	46	..	..

* The terms "before" and "during" are used to indicate that the collections of urine were made at comparable periods and intervals, following a standard breakfast, although no massage was given.

The analytic data are expressed in terms of quantities excreted per hour for the period preceding the massage and for the period during and immediately following the massage.

In table 1 are given the results of twenty experiments in which the effect of massage on the composition of the urine was studied. Abdominal massage in addition to the general massage was given in seven of the experiments (14 to 20).

The massage consisted of a vigorous general muscular massage of from thirty to sixty minutes' duration, given by a trained masseur, or masseuse. The subjects were patients who had arthritis and patients convalescing from operations. Nearly all of them were accustomed to some form of massage, therapeutically given, and consequently were suitable for receiving vigorous treatment.

In table 2 are ten control experiments performed on days when no massage was given. The specimens of urine were collected under the same dietary regimen and at the same time intervals as prevailed during the massage experiments. The subjects were the same persons who had served for the previously described experiments. The control and massage experiments were usually carried out on successive days.

RESULTS

Examination of the tables reveals clearly that despite the standardization of the breakfast following a fourteen hour fast, and the conduction of the experiments under uniform conditions of time and procedure, the influence of the subject's previous diet was not eliminated. The wide variations exhibited in the hourly excretion of certain of the urinary constituents and the lack of uniform behavior during the premassage periods would seem to be ascribable, in part at least, to differences in the individual dietary habits.

These results show no marked change in the composition of the urine that can clearly be ascribed to the effect of the massage, although there are indications in some cases that the massage has had an effect on renal activity. An increase in volume of urine secreted per hour was found more frequently following massage than during the control periods. This was especially true when abdominal massage was given. Occasionally a true diuresis was observed. Increased intra-abdominal pressure accompanies abdominal massage, and this may in turn cause a diuresis. Bazett and his co-workers [13] have reported diuresis following the application of abdominal pressure, and they suggest that the diuresis which they observed during baths was caused by the pressure of water on the abdomen, which in turn influenced the venous blood pressure.

No effect was observed on the secretion of acid which could be ascribed to massage. This is in keeping with previous work [1] in which it was shown that massage produced no disturbance in the acid-base equilibrium of the blood, differing in this respect from exercise.

An increased rate of excretion of nitrogen, inorganic phosphorus and sodium chloride was found during the massage period more often

13. Bazett, H. C.; Thurlow, S.; Crowell, C., and Stewart, W.: Am. J. Physiol. **70**:430 (Oct.) 1924. Griffith, J. Q., and Hansell, H. R.: Am. J. Physiol. **74**:16 (Sept.) 1925.

than during the corresponding period in the control experiment. The increases in these constituents frequently are not large and do not always accompany massage.

Massage has no effect on the rate of excretion of creatinine, judging from the results of the massage and control experiments. In one case only was a definite creatinuria observed, and in this instance the rate of creatinine excretion was not changed during massage. In none of the subjects was a creatinuria induced by the massage. In our hands the method for the determination of creatine was hardly adequate to detect small quantities.

Taken as a whole, these results fail to show any marked changes of the rate of excretion of urinary constituents during vigorous muscular and abdominal massage. The benefit of massage seems, then, to depend on broad physiologic effects rather than on its influence on any specific end-products of metabolism. Rosenthal,[2] after reviewing the physiologic effects of massage, believes that they may be explained by an increase in the blood flow through the parts massaged. That the benefits accruing from massage probably result from changes in the circulation has also been suggested on other grounds by Pemberton, Cajori and Crouter.[1] The experimental results recorded in this paper further substantiate this view.

SUMMARY

1. The rate of excretion of water, acid, total nitrogen, sodium chloride, inorganic phosphorus, creatinine and creatine was ascertained in a group of subjects following a standard breakfast. The effect of vigorous muscular and abdominal massage, given under the same conditions, was also determined in respect to the rate of excretion of these urinary constituents.

2. An increased volume of urine frequently accompanied massage, and at times a definite diuresis was observed. This was especially true when abdominal message was given.

3. Accompanying or following massage an increase was frequently, but not always, observed in the rate of excretion of total nitrogen, inorganic phosphorus and sodium chloride.

4. No evidence that massage influences the reaction of urine or the excretion of creatinine or creatine was obtained.

5. It may be concluded from these experiments that massage has no immediate or great influence on general metabolism per se. The cumulative effect which massage nevertheless exercises on various metabolic processes probably lies in its mechanical influence on the circulation of the parts concerned.

We are indebted to Mr. J. J. O'Brien, Miss Blanche Henninger and Miss Hazel John for their willing and essential cooperation in carrying out the massage.

THE ACOUSTICS OF THE BRONCHIAL BREATH SOUNDS

APPLICATION TO PHENOMENA OF AUSCULTATION AS HEARD IN LOBAR PNEUMONIA *

GEORGE FAHR, M.D.
MINNEAPOLIS

It is usually taught that the typical signs of lobar pneumonia are bronchial breathing, bronchophony and increased tactile fremitus, but the onset of a lobar pneumonia is rarely associated with bronchial breath sounds, bronchophony and increased tactile fremitus, the classical signs of a lobar pneumonia as obtained by auscultation. The inspiratory breath sounds are usually vesicular in quality but much reduced in intensity or entirely absent at first. They not only are faint but also sound as if they came from a greater distance than normally. The expiratory breath sound may be fainter than the normal or slightly increased in intensity and duration. At this time not only is there no bronchophony present, but the transmission of the spoken voice may be much reduced and the tactile fremitus may be absent or reduced in intensity over the area of pneumonia. This period of faint and distant vesicular breath sounds may last only a few hours, or it may last a number of days. During the last year, I have had occasion to study carefully about forty cases of pneumonia. It was not common to find bronchial breathing, increased tactile fremitus and bronchophony present over the area of pneumonia during the first twenty-four to forty-eight hours. The majority of the patients exhibited faint and distant breath sounds of vesicular character, with decreased fremitus and transmitted voice over the area of pneumonia for from twenty-four to seventy-two hours. During this period the roentgen ray might show only a slight haziness over the area and not a dense shadow, or it might show a moderately dense shadow. When the breath sounds became distinctly bronchial, and when tactile fremitus increase and bronchophony were present, the roentgen rays always showed a dense shadow over the area of pneumonia.

The following two cases are illustrative:

The patient developed a chill and stitch in the right side and pain over the abdomen; an acute appendicitis was diagnosed. Fever and leukocytosis of 30,000 were present. Forty-four hours after the onset of the pain, I was called in by the surgeon who had been asked to operate to decide concerning the presence of pneumonia. The surgeon had found the abdominal symptoms slight and could not easily reconcile an acute appendicitis with the leukocyte count of 30,000. Suspecting pneumonia, he had made a physical examination but felt that the physical signs were not convincing.

* Read by invitation before the Lymanhurst Medical Staff, March 23, 1926.
* From the Department of Medicine, University of Minnesota Medical School.

I found the patient exhibiting only mild dyspnea, but there was definite restriction of movement over the lower part of the thorax on the right side during respiration. Percussion revealed a high pitched, somewhat hyperresonant note over the right lower lobe. Auscultation revealed an occasional crepitant râle over this right base. The inspiratory breath sounds over the right lower lobe were faint—they seemed to come from a distance greater than normal—and the expiratory murmur was slightly, but definitely, prolonged and certainly not diminished in intensity. Tactile fremitus was absent over the right lower lobe, and the spoken voice was poorly transmitted, being less intense than on the left side. A diagnosis of lobar pneumonia involving the right lower lobe was made, and transfer to the medical service was advised. The intern assigned to the case was skeptical as to the diagnosis but the following morning, seventy-two nours after the onset, reported that bronchial breathing, increased tactile fremitus and bronchophony were present. His physical examination of the patient was confirmed, and the requested roentgen-ray examination resulted in a report of "lobar pneumonia involving the right lower lobe."

In a case recently seen, breath sounds and tactile fremitus were absent on the second day of pneumonia, at which time there was intense dulness over the whole right lower lobe and part of the left lower lobe. The physical signs suggested fluid in the chest; yet on the third day intense bronchial breathing and bronchophony were present over the dull area, and tactile fremitus was intense. This is typical of my experience with the physical signs of lobar pneumonia.

In order to understand the physical signs of pneumonia it is necessary to review our knowledge of the physics of sound and to apply it to the problems of the production and transmission of sounds in the tracheobronchial system under normal and pathologic conditions. For this purpose, sounds must be considered as sensations in consciousness brought about by vibrations of the air which is in contact with the ear drum. In the case of the normal and abnormal breath sounds and spoken voice sounds, these vibrations are produced within the tracheobronchial system and are transmitted to the thorax wall along the walls and within the walls of this system. From the thorax wall the vibrations are conducted to the observer's ear drum through the lumen of his stethoscope. The vibrations are not simple vibrations of a single frequency or pitch but are made up of a number of vibrations of different frequencies. The higher the frequency of a vibration, the higher the pitch as perceived by the observer. Physicists speak of the vibration of lowest frequency or pitch as the fundamental, and the vibrations of higher pitch as the overtones, in a sound compounded of vibrations of various frequencies. A given instrument for the production of sound will usually produce a sound made up of more than one frequency. The lowest frequency is the fundamental for that instrument and is usually much the loudest vibration produced by the instrument. As a rule, the overtones of any given instrument are less intense than the fundamental tone of the instrument.

The trachea and the bronchial tree compose a system of tubes of varying length and internal diameter connected with each other and with rigid walls down to the bronchi of about 2 mm. diameter. The laws and theories of physics that relate to the production of sounds in pipes

and the transmission of sound waves through tubes must therefore be applied to the problems of the production and conduction of the bronchial breath sounds in the lungs of man, if the difference in the physical signs at the onset and later in the disease is to be understood.

There are two kinds of pipes so far as the physics of sound production is concerned; namely, the reed pipe and the labial pipe. In the reed pipe, a vibrating tongue or membrane set into motion by a stream of air produces a sound the pitch of which is partly determined by the dimensions and elastic coefficient of the vibrating part and partly determined by the dimensions of the pipe. The pitch of a reed pipe is prac-

Fig. 1.—Schematic cross-section of labial organ pipe showing pendulating air current as demonstrated by smoke in issuing air current. In the tracheobronchial system the sharp edge at the points of bifurcation functions during inspiration as a lip in a labial pipe. (From Myers and others "The Normal Chest," to be published by Williams & Wilkins Company, Baltimore, 1926.)

tically invariable when the velocity of the air blowing the reed is increased. Air under pressure issues from a narrow opening and strikes against a sharp edge in the typical labial pipe. The issuing air column is thrown into vibration about the sharp edge (fig. 1), and vibrations of this air column which are of the same pitch as the fundamental tone of the pipe or one of its overtones are reinforced in intensity by resonance with the column of air in the pipe, and a sound is secured the pitch and quality of which are determined largely by the dimensions and material of the pipe.

If one blows through a glass T tube, a sound is produced. The air issuing from the side tube at high velocity is set into vibration both as

it leaves the side tube and emerges into the other tube and as it strikes against the wall of the other tube. Vibrations whose frequency corresponds to the fundamental frequency of the tube in which the vibrations have been produced set the air in this tube vibrating at this frequency, and a sound is produced the pitch of which corresponds to the fundamental frequency or pitch of the tube into which air has been blown. The same sound is produced if the two tubes do not meet at a right angle (fig. 2). Tubes such as this are to be classed with the labial pipes so far

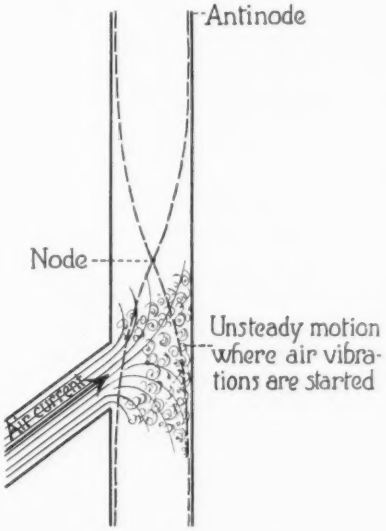

Fig. 2.—Schematic representation of the conditions in the tracheobronchial system during expiration. Air moving through the side branch with considerable velocity emerges into the main tube and is set into vibration as it leaves the side branch and also as it strikes on the opposite wall of the main branch. This sets up vibrations of the air in the main branch of a frequency corresponding to its fundamental pitch. At the ends of the main tube there are antinodes and in the middle nodes according to the well known law of open pipes.

as sound production is concerned, for the mechanism of the sound production is practically the same as that in the true labial organ pipe. The pitch of the sound produced by a labial pipe is not invariable as in the reed pipe, for if the velocity of the stream of air blowing the pipe is increased sufficiently, a point is reached at which the pitch of the sound produced jumps up one whole octave; if the speed of the stream of air is increased still further, a velocity is reached at which the pitch jumps another octave. The velocity of the air blown into a labial pipe can then, to a certain extent, alter the pitch of the sound, and this alteration is always of the nature of one or two octaves' increase in pitch.

The first problem is to determine whether the bronchial breath sounds are produced by a reed pipe or by a system of labial pipes, and then to determine where this pipe or these pipes are located in the bronchial tree. Bushnell [1] has maintained that the bronchial breath sounds are produced by vibrations set up in the vocal cords by the passage of air through the vocal cords, and that the intensity and character of the sound is modified by resonance in the trachea and bronchial tubes; in other words, Bushnell believes that the tracheal and bronchial breath sounds are produced by a reed pipe system. Many simple experiments show that this cannot well be the case. If a stethoscope is placed over the trachea or larynx and air is breathed in and out at normal velocity, a tracheal tone is heard which has the normal pitch of the tracheal tone. If the observer now blows air out under extreme exertion, thus increasing the velocity of the column of air, he can get the pitch to jump one octave or even two. I have had two competent musicians auscultate my own tracheal tones during normal expiration and during extremely forced expiration, and they both assured me that the pitch jumped one octave on forced expiration. On one occasion, one of the musicians heard it jump two octaves. This experiment seems to show that the tracheal tone is produced by a labial type of pipe.

Martini,[2] who deserves the credit for emphasizing the labial pipe character of the tracheobronchial breath sounds, has auscultated the tracheal breath sounds over the vertebra prominens after passage of a tracheoscope so that the vocal cords could play no part in the production of the sound, and he has found the tracheal tone present and little altered. I have auscultated the tracheal tones over the trachea and vertebra prominens, and the bronchovesicular breath sounds over the right apex, in a number of persons before and after the passage of the bronchoscope, and I have found only a little change in the breath sounds. Recently, I had the opportunity to auscultate a patient who had had the larynx removed surgically. He breathed through a tracheotomy tube inserted into the trachea about 2 inches (5 cm.) below the point where the larynx had been cut off. The breath sounds everywhere were normal. The tracheal sounds auscultated over the vertebra prominens were normal, and could be heard with diminishing intensity down to the third dorsal spine. Their pitch was higher than that of the average normal person and the intensity was somewhat less than that of the average normal person. The bronchovesicular breath sounds in the second right interspace were bronchovesicular in character in this patient. The breath sounds over the right apex in the

1. Bushnell and Pratt: Physical Diagnosis of Diseases of the Chest, Philadelphia, 1925, p. 37.

2. Martini: Studien ueber Perkussion und Auskultation, Habilitationschrift, München, 1922.

supraclavicular fossa closely approached bronchial breath sounds in character. These sounds were less intense and slightly higher in pitch than in the average normal person; yet on auscultating a large number of normal persons, I could find some whose breath sounds in this area resembled those heard in the patient without a larynx. A complete survey of the breath sounds in this patient over all areas resulted only in determining that the pitch of the tracheal and bronchial breath sounds was higher than in normal persons and probably less intense. The glottis, therefore, plays only a minor part in the production of the tracheal and bronchial breath sounds.

Bullar [3] has shown on exenterated calf lungs that the inspiratory vesicular murmur is formed in the air sacs themselves, and that the bronchial breath sounds are formed largely in the trachea and larger bronchi. In his experiments, the left lung was fitted air tight into an artificial thorax and the right lung was outside the thorax in a state of massive collapse. The two lungs were connected by the trachea, which was cut off below the larynx. A bronchial breath sound was heard over the right collapsed lung outside the thorax when inspiration was produced in the left lung by lowering the pressure outside this left lung in the artificial thorax. When a plug was inserted into the trachea, and when air was forced out of the left lung and into the right lung by increasing the pressure in the artificial thorax outside the left lung, a vesicular inspiratory murmur was heard over the right lung; in other words, when air entered the air sacs of the right lung, a vesicular inspiratory murmur was heard. These experiments prove that the glottis plays a minor part in the production of the bronchial breath sounds. It may be concluded from the foregoing experiments that the tracheobronchial breath sounds are produced by labial pipes, because vibrations of the glottis play no important part in the production of the bronchial breath sounds, and it contains the only available membranes for the production of sound within the tracheobronchial tubing.

During inspiration the mechanism of formation of the inspiratory bronchial breath sound corresponds closely to the mechanism of the organ pipe in figure 1. The air current striking on the bifurcation points of the tracheobronchial system is set into vibration at these sharp edges, and the resulting sound has the pitch of the tube in which these vibrations are present. During expiration the mechanism is largely as represented in figure 2. As air emerges from a smaller system into a larger one, it is set into unsteady motion or vibration, and these vibrations set up sound vibrations of pitch corresponding to the dimensions of the larger tube into which the air is emerging.

3. Bullar: Experiments to Determine the Origin of the Respiratory Sounds, Proc. Roy. Soc. London **37**:441, 1884.

The various tubes making up the bronchial tree are of different lengths and diameters and therefore will have different fundamental periods of free vibration or pitch. If the length of an open pipe is doubled, the pitch of the fundamental is reduced one octave. The width of a pipe has a certain influence on its pitch also in that a diminution in diameter tends to lower the pitch of the fundamental. If the walls of a pipe become less rigid, the pitch becomes lower and the sound character or timbre changes somewhat. It is therefore logical to suppose that the bronchial breath sounds contain vibrations of different frequencies, and it is of interest to know what these frequencies are and what bronchi are concerned in their production.

Martini and Mueller [4] have done an excellent piece of work on the production and conduction of the bronchial breath sounds. Martini [4] had shown by graphic methods that the bronchial breath sounds in two patients examined by him had a pitch between c^1 and d^2, that is, a frequency between 320 and 540 vibrations a second. By means of resonators he determined that the bronchial breath sounds also contained overtones of approximately 1,000 vibrations a second, or around c^3 on the musical scale. Cabot and Dodge [5] have shown by means of the stethophone that the lowest frequency of the bronchial breath sounds lies between 240 and 400 vibrations a second, and that the highest lies between 660 and 1,000 vibrations a second. Working with the original model of the stethophone in Dr. H. B. Williams' laboratory, department of physiology, Columbia University, I found the tracheal breath sounds largely composed of vibrations between 400 and 660 vibrations a second. A not inconsiderable portion of the sound energy consisted in vibrations between 660 and 1,000 a second. A small amount of the sound energy consisted of vibrations between 120 and 400 a second.

With the knowledge that the bronchial breath sounds have a fundamental of about 300 to 500 vibrations a second, and that they contain overtones of a frequency around 1,000 a second, Martini and Mueller determined to find just what parts of the tracheobronchial system of labial pipes could produce vibrations of this frequency. They first recorded graphically the vibrations set up in the system of tubes comprised of the mouth, nasal cavities and tracheobronchial system of a person connected by a glass T tube in the mouth to a recording optical capsule on the one side and closed by a condom rubber on the other side of the T tube. When the rubber membrane was burned through, a number of free vibrations of the system were produced, and these were recorded by the optical capsule on a photographic drum. The free vibra-

4. Martini and Mueller: Studien Ueber das Bronchialatmen, Deutsches Arch. f. klin. Med. **143**:159, 1923.

5. Cabot and Dodge: Frequency Characteristics of Heart and Lung Sounds, J. A. M. A. **84**:1793 (June 13) 1925.

tions of this system were investigated in five persons. The fundamental frequency of this system recorded in this way varied between 400 and 600 vibrations a second. They then proceeded to measure the fundamental frequency of the tracheobronchial system below certain levels. In order to do this, it was necessary to make use of lungs taken from the bodies of patients who had recently died. Then the system was cut through at various levels and the T tube, with recording optical capsule on one arm and the condom membrane on the other, was inserted into the distal end of the cut bronchus. When the condom membrane was

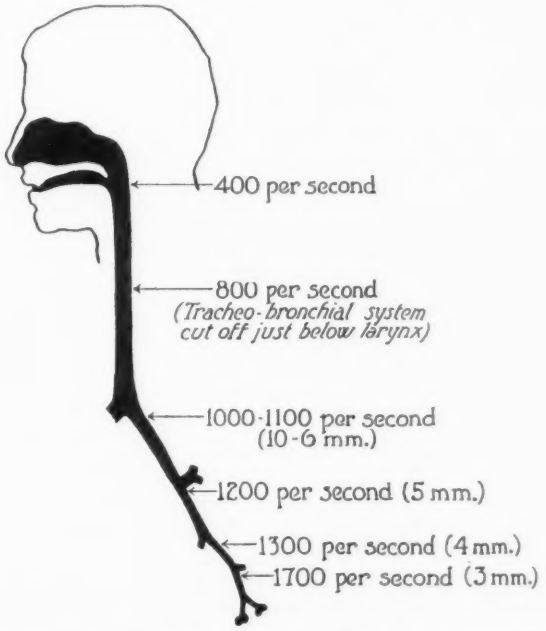
400 per second
800 per second
(Tracheo-bronchial system cut off just below larynx)
1000-1100 per second
(10-6 mm.)
1200 per second (5 mm.)
1300 per second (4 mm.)
1700 per second (3 mm.)

Fig. 3.—Schematic longitudinal section of tracheobronchial tree with frequency of free vibrations of component tubes as determined by Martini and Mueller (Deutsches Arch. f. klin. Med. **143**:159, 1923).

burned through, the air in the bronchus was given an impulse, and it vibrated in its own free period or frequency; these vibrations were recorded by the optical capsule on the photographic kymographion.

Figure 3 gives the approximate fundamental frequency of various parts of the tracheobronchial system of adults as determined by Martini and Mueller. The recording T tube was inserted into the bronchus of the diameter shown at various levels, in figure 3, and vibrations of approximately the frequencies inserted in the drawing at these levels were set up in the system by burning through the membrane. In

this illustration it is shown that the tracheal system obtained by cutting off the trachea just below the larynx and inserting the recording apparatus in the distal end has a fundamental frequency of 800 vibrations a second. The bronchial system obtained by cutting off a main bronchus just below the bifurcation has a fundamental frequency of 1,000 vibrations a second. As the periphery is approached, the fundamental pitch of the system becomes higher. The fundamental frequency of the free vibrations of the system is 1,700 a second, if the recording apparatus is inserted into a bronchus of 3 mm. internal diameter. Therefore it can be asserted that the bronchial tubes of 3 mm. internal diameter or less take no part in the formation of the audible bronchial breath sounds because the lowest free vibration in them is of a pitch much higher than the highest frequency of bronchial breathing as recorded by Martini over areas of consolidation. The fundamental frequency of the system from 4 mm. internal diameter peripheriward is also too high to take part in the formation of audible bronchial breath sounds.

From Martini and Mueller's investigations and the acoustic principles previously discussed here it may be concluded that the tracheobronchial breath sounds are formed by the movement of air columns through the tracheobronchial system during breathing, whereby pendulating air movements are formed at bifurcation points and where narrow cross-sections emerge into wider ones. These pendulating air movements are increased in intensity by resonance in the various portions of the pipe system down to bronchi of an internal diameter of from 4 to 5 mm. These intensified air vibrations are the physical basis of the sounds. The fundamental vibration or pitch of the bronchial breath sound is formed in the mouth, nasal cavities and larynx. The overtones which give the sound its characteristic "Klang" are formed in the larger bronchi down to a lumen of 4 to 5 mm. diameter.

The sound vibrations produced in the tracheobronchial system must be conducted to the chest wall in order to be auscultated by the physician. The greater part of the sound energy is conducted through the air columns of the bronchial tube system much as the sounds are conducted through the air columns of a stethoscope. A small part of the sound energy enters the walls of the bronchi and the connective tissue in contact with these walls and is conducted along these walls toward the periphery.

Whenever sound vibrations of a given frequency or pitch are impressed on a vibrating system of lower frequency or pitch, there is considerable loss in the amplitude of vibration or intensity of the sound; on the other hand, if the frequency of the second system is greater than that of the first, there is little loss in amplitude or intensity. Therefore as the sound is transmitted along from one system of tubes to the next

with smaller internal diameter but higher frequency, there is little loss of energy from the forcing of the vibration of slower frequency on the system of greater frequency. On the other hand, when the sound reaches tubing of a diameter of 5 mm. or less, it is reduced rapidly in intensity because of the friction of the sound in small tubes, as Martini [6] has shown for the conduction of sounds in stethoscope tubing. Schulze [7] has shown that the friction in tubes of 2 mm. internal diameter is enormous, and that it is practically impossible to force sound waves through tubing of 1 mm. internal diameter. Martini [6] has shown that when a loud sound is sent through a tube of 3 mm. internal diameter and only 4 cm. length, the intensity is greatly diminished. Under these circumstances, the tracheobronchial breath sounds are not conducted within the tubes farther toward the lung periphery than to the point where the internal diameter of the tubing is 3 mm. At this point most of the sound energy is dissipated as heat through friction.

A small part of the sound energy is conducted along the walls of the bronchi and the connective tissue toward the pleural surface of the lung. This sound energy must to a large extent diffuse through the air sac containing portion of the lung whose fundamental frequency has been determined by Martini to be about from 100 to 150 vibrations a second. The bronchial breath sound vibrations are all much more frequent than this, and much of the intensity of these vibrations is lost when they are necessarily impressed on a system whose vibration period is much slower. It has also been shown experimentally by Sahli, Martini, Montgomery and others that porous lung substance is a poor conductor of the sounds of tracheal breathing. If a piece of normal lung is placed over the trachea, the tracheal sounds are barely heard. Substances like porous lung are good absorbers of sound, whereas consolidated lung is a good conductor of sound vibrations. If a piece of consolidated lung is placed over the trachea, it will be found that the tracheal sounds are fairly well transmitted through the consolidated lung tissue.

Bronchial breath sounds or bronchovesicular breath sounds are heard over the right apex, where large bronchial branches come close to the surface. The small volume of porous lung between these large branches does not completely absorb the bronchial breath sounds, and both faint bronchial breath sounds and the inspiratory vesicular murmur are heard over these areas. The faint, often inaudible expiratory breath sound over areas of the lung is the only part of the bronchial breath sounds that is transmitted to the thorax over these areas.

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6. Martini: Die Schalluebertragung des Stethoskops, Ztschr. f. Biol. **53**:117, 1920.

7. Schulze: Ueber die Schalgeschwindigkeit in sehr engen Roehren, Drudes Ann. der Physik, ser. 4, **13**:1060, 1904.

The inspiratory breath sound is associated with air entering the alveoli, as was shown by Bullar's [3] experiments, mentioned above, and by Sahli,[8] who auscultated a patient with hernia of the lung when the glottis was closed. During expiratory effort under these circumstances, an inspiratory breath sound was heard over the herniated lung, whose air sacs filled during the expiratory effort with a closed glottis.

In order that the typical bronchial breath sounds may be heard, there must be a good conducting contact between the chest wall and bronchi of 3 mm. internal diameter or greater. Then the sound waves coming down along the bronchial tubes impinge on the good conducting material. At the surface of the good conducting material, some sound energy is lost by reflection but much is transmitted with only moderately diminished intensity to the thorax wall, where the physician can pick it up with his stethoscope. Consolidated lung tissue is a good conductor of the sounds of bronchial breathing, and normal lung tissue is a poor conductor of sounds of this frequency, as stated in the foregoing. The consolidation must reach from the pleura inward to bronchi of 3 mm. diameter or larger, if bronchial breathing is to be heard. The intensity will be greater if the consolidation reaches farther toward the hilum, for then good conducting contact is made with larger bronchi, and the sound energy has been even less dissipated on its way along the tubes. Martini and Mueller [4] have shown that bronchi of 3 mm. internal diameter are about 3 cm. inward from the pleura in the upper portions of the lung. Bronchi of this caliber are found 3.5-5 cm. hilumward from the pleura in the basal and axillary portions of the lung. Bronchi of this bore reach to within 1 to 2 cm. of the pleura near the vertebral column. These measurements on cadaver lungs give a good idea of how far a consolidation must extend from the pleura toward the hilum before the faintest bronchial breathing is heard in pneumonia.

The voice sounds are produced by vibrations of the vocal cords, and are modified in intensity and timbre by resonance in the mouth and nasal cavities. These sounds are composed of vibrations of various frequencies, but there are certain characteristic frequencies present in all vowel and consonant sounds no matter what the fundamental pitch is. These frequencies, called formatives, are what characterize the vowel and consonant sounds for our ears. When they are absent, the vowels and consonants do not appear to us to be the vowels and consonants of our spoken language. All the formatives of the vowels and consonants have a pitch much higher than the natural frequency of the porous lung substance (from 100 to 150 a second), with the exception of the formative of *oo*, which has the pitch of *f* on the musical scale and a frequency of 170 vibrations a second, and one of the formatives of *ee*, which has the

8. Sahli: Ueber die Entstehung des Vesiculaerathmens, Cor. Bl. f. schweiz. Aerzte. **22**:265, 1892.

same pitch. The voice sounds are conducted down the lumen of the bronchial tubing as well as in the walls of the tubing and the connective tissue surrounding these walls. Practically all the energy of the sounds conducted as plane waves within the tubing is dissipated as heat energy when it enters tubes of 3 mm. internal diameter. No sound energy within the tubes gets farther toward the periphery than tubes of 1 mm. internal diameter. When auscultation is performed over normal lung, none of the sounds that are conducted within the tubing are heard; but the sound conducted along the walls of the tubing and the interstitial connective tissue gets to the neighborhood of the alveoli with relatively little diminution of its energy. The frequency of the porous lung substance, as Martini [6] has shown, lies between 100 and 150 or thereabouts. Sounds of this frequency may be made more intense by resonance in

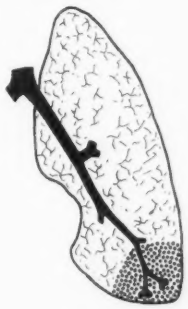

Fig. 4.—Schematic representation of tracheobronchial tree and lung with lobar pneumonia extending inward to bronchial tubes of 3 mm. internal diameter. Represents necessary condition for conduction of bronchial breath sounds to thoracic surface.

the tissue. Sounds of lower frequency lose little energy when impressed on this system of higher frequency. Sounds of higher frequency lose energy when impressed on systems of lower frequency, and the farther removed a frequency of higher vibration is from the frequency of the slower system, the greater the loss in intensity of the energy of the more rapid vibration when it is impressed on the slower system. For this reason alone the porous lung substance is a poor transmitter of sounds of pitch above *f* on the musical scale, or 170 vibrations a second. In addition, substances with small air holes dissipate much sound energy through friction in the small pores. Therefore, only a little of the voice sound energy reaches the thorax wall, and of this energy only the smallest part is of high pitch. The characteristic formatives of the spoken voice sounds are with few exceptions of pitch higher than 170; therefore, the words heard over normal lung are indistinct.

Any physician can test out part of these conclusions by having some one with a bass voice sing the notes *c* (130 vibrations) or *f* (170 vibrations) while he palpates the fremitus; then if the singer sounds c^1 or f^1 or any other note of much higher pitch with the same intensity, the fremitus becomes much less intense. It is hard to get a good, strong vocal fremitus over the lung of women with soprano voices because their lowest notes are above the natural period of their lungs. Part of the energy of the voice sounds conducted down the lumen of the bronchial tubes is reflected and part of the energy of these sounds enters a consolidation, which reaches from the periphery of the lung inward toward the hilum to areas where bronchi of internal diameter of 3 mm. are present. This energy is conducted with little loss to the thorax wall except by diffusion, according to the acoustic law of inverse square of the distance from source in isotropic mediums. In addition, the energy conducted along the bronchial walls and connective tissue enters the consolidation and is well conducted to the thorax. A loud sound in which the words are not distinct is heard, and a strong fremitus is felt under these conditions. The words are indistinct because in conduction along the lumen of tubes from 5 to 6 mm. bore down to the tubes of 3 mm. bore much sound energy is dissipated by friction, and as more of the energy of sounds of higher pitch is lost in friction, the formatives and characteristic overtones are relatively much less strong than in the sound as spoken; therefore, the words are indistinct. The sounds are fairly distinct, and pectoriloquy is present when the consolidation reaches inward to bronchi of 5 to 6 mm. bore.

The conclusions have been confirmed experimentally by Martini in his "Habilitationschrift." He recorded vowel sounds as spoken into his optical recorder and as obtained from the chest wall over normal and consolidated lung. A curve of sound vibrations of fundamental frequency 240 and an overtone of 960 vibrations a second was recorded when the vowel German *u* was spoken directly into the recording instrument (fig. 4 b). The sound of 960 vibrations is the formative of German *u*, as determined by Helmholtz many years ago.

The sound as recorded from the chest over normal lung shows only the vibration of 240 a second; the formative has disappeared (fig. 5 d). In addition, the intensity of this vibration is much less over the normal chest. The sound recorded over a pneumonic consolidation in the same person shows again the frequency of 240 and 960 vibrations a second, and the relative intensity of the formative is nearly what it was in the sound spoken directly into the instrument (fig. 5 c). In addition, the intensity of the fundamental is greater over the consolidation than over the normal lung. We have in this instance an example of pectoriloquy so far as the spoken vowel German *u* is concerned.

Most types of pneumonia begin near the pleural surface, are associated with a pleurisy and progress inward toward the hilum more or less rapidly. A few types of pneumonia in adults may develop centrally and proceed outward toward the pleura. I shall first discuss the types of pneumonia which begin near the pleura. The first stage is a hyperemia soon followed by an exudation of relatively limpid serous fluid. As a rule, the movements of the chest over this area are limited even at this stage. Therefore, less air enters the patent alveoli, and the inspiratory breath sound must be less intense. The serous fluid soon

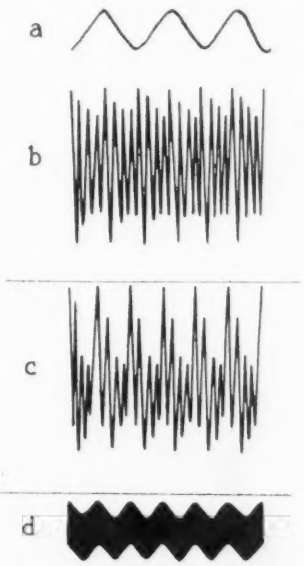

Fig. 5.—*a* indicates tuning fork vibrations of 110 complete vibrations a second. *b*, photographic record of fundamental and formative of German "u" spoken directly into recording apparatus. The fundamental frequency is 240 vibrations a second. Frequency of formative is 960 a second. *c*, photographic record of vibrations over pneumonic consolidation when German "u" is spoken. Formative well represented but relatively less intense than in *b*. *d*, photographic record of vibrations over normal lung of same person when German "u" is spoken. Formative is absent and fundamental is much reduced in intensity. (Reproduction of Martini's curve taken from Martini Studien Ueber Perkussion und Auskultation, Habilitationschrift, München, 1922.)

becomes more viscous and finally coagulates. The different alveoli will contain fluid in various degrees of viscosity. In some it will be limpid; in others, viscous, and in still others, it will be solid. At this stage crepitant râles will be heard, for they are formed when fluid is present in the finest air passages and in the air sacs themselves. When the fluid gels, crepitant râles cannot be formed. The inspiratory vesicular

murmur becomes faint or disappears entirely, for little or no air enters the air sacs in the pneumonic area now. The expiratory murmur, which is the last rest of the expiratory bronchial breath sound conducted out along the bronchial walls and interstitial tissue, often becomes fainter because when these sound waves enter the pneumonic area, much energy is lost in reflection. The consolidated lung is already a better conductor of sound than the normal porous lung, but because of the various degrees of fluidity of the exudate, and because there may still be some air in the air sacs, many chances for reflection of sound energy are given at the boundaries of materials of different specific gravities and different coefficients of elasticity. For the same reason, the intensity of the spoken voice and tactile fremitus become less in this stage. The energy of the spoken voice sounds is partly reflected at boundaries of different specific gravity and rigidity, and therefore the energy transmitted to the thoracic surface is reduced.

Bronchial breath sounds and bronchophony appear, and tactile fremitus increases in intensity when the consolidated lung reaches inward from the pleura to areas where direct contact is made with bronchi of 3 mm. internal diameter or greater. Under these conditions, there is also some sound energy lost by reflection at the boundary of the consolidation, and less is lost when the exudate is coagulated completely. But more of the energy of the breath sounds and voice sounds is now conducted to the pleural surface, because not only part of the energy of the sound conducted along the walls and interstitial connective tissue is transmitted but also part of the much more intense sound energy within the tubing. It is the latter energy which really determines the audibility of the bronchial breath sounds. As the consolidation proceeds inward, it reaches bronchi of 5 to 6 mm. bore; the bronchial breath sounds become intense, and pectoriloquy begins to appear, for friction in the tubes has not destroyed much of the energy of the sounds conducted through them until bronchi of this lumen are reached.

Bronchial breath sounds are heard, and bronchophony and tactile fremitus are increased over large volumes of fluid in the chest when this fluid compresses the porous lung substance down to direct contact with bronchi of about 3 mm. bore. Under these circumstances the physical signs over fluid are much like those over consolidation, and not infrequently massive exudates in the chest are diagnosed as consolidation when these physical signs are present.

The intensity of the expiratory breath sound varies not a little during the progress of the consolidation from the pleural surface inward. If the pneumonic process is consolidated completely, the exudate firmly gelled, the expiratory breath sound may be prolonged and intense because there is reflection only at one surface and the intensity of the expiratory breath sound has lost less by absorption in the porous lung

substance, because it now passes through less of this porous substance. Moreover, the inspiratory murmur though faint may be suggestive of the bronchial inspiratory breath sound because a small amount of it reaches the consolidation along the walls and interstitial tissue, there being less absorption than in the normal lung, and not a great deal of loss through reflection at the boundary of the consolidation. The breath sounds are then bronchovesicular.

If the pneumonia starts near the hilum, bronchial breath sounds will be heard only when the consolidation reaches the periphery. The same may be said for bronchophony and tactile fremitus. I should assume on theoretical grounds that there would suddenly be intense bronchial breathing and pectoriloquy when the consolidation reached the pleural surface, whereas previously the breath sounds would have been faint, and the voice sounds transmitted normally. There should not be the same gradual transition that obtains when the pneumonic process proceeds hilumward from the pleura. I have never been able to test my assumption experimentally, for I have never seen what I could convince myself was a real central pneumonia during the years that I have been interested in the interpretation of physical signs on a physical basis.

It has often been assumed that absent bronchial breath sounds, tactile fremitus and bronchophony could be explained by a supposed mucous plug in a large bronchus leading to the area. Such a plug would probably lead to the condition of massive collapse, in which, as we know, bronchial breath sounds and increased tactile fremitus may be present. Moreover, this assumption is not necessary, for the absence of bronchial breathing and bronchophony and decrease in tactile fremitus can be explained on the basis of the known facts of pathologic changes and their acoustic consequences. On the other hand, in some cases of lobar pneumonia there is a thin layer of fluid over the area of consolidation, and this fluid may aid in the suppression of the breath sounds, bronchophony and tactile fremitus.

CONCLUSIONS

Bronchial breath sounds are formed by the movement of air columns through the bronchial tubes from the larynx to the bronchi of 5 mm. internal diameter. The manner of formation is essentially that for the formation of sounds in labial pipes.

The nose, mouth and larynx condition vibrations of a frequency of 400 to 600 a second. The trachea conditions vibrations of a frequency of 800 per second. The main bronchi condition vibrations of 1,000 per second.

These sounds are conducted along the lumen and in the walls and surrounding connective tissue of the walls toward the periphery of the chest. The sound within the lumen is nearly completely obliterated by

friction when tubes of an internal diameter of about 2 mm. are reached. Only part of the sound energy of the expiratory bronchial breath sound reaches the thorax wall, being conducted along the walls of the tubes and through the strongly absorbing porous substance of the alveolar structure.

Bronchi with an internal diameter of 3 mm. are 3 cm. from the pleural surface in the upper lobes and 3.5 to 5 cm. from the pleural surface in the lower lobes except near the vertebral column where they are only from 1 to 2 cm. from the periphery.

Consolidation must reach inward this far in order to make good conducting contact with bronchial tubes of 3 mm. bore and thus conduct bronchial breath sounds to the wall of the thorax in pneumonia.

The first physical signs of pneumonia obtained on auscultation are: crepitant râles, diminished or absent breath sounds and diminished spoken voice. Tactile fremitus is also diminished at a time when the consolidation has not reached bronchi of 3 mm. internal diameter.

There are bronchial breathing and bronchophony and a stronger fremitus when bronchi of this diameter are surrounded by consolidation reaching to the periphery.

Pectoriloquy appears when the consolidation reaches in to around bronchi of 5 to 6 mm. internal diameter.

The spoken vowel sound over normal lung area contains only the fundamental frequency of the vowel sound. The condition of pectoriloquy is obtained when the vowel sound is so well conducted to the chest wall that the sound contains the formatives as well as the fundamental frequency of the vowel sound. Bronchophony is the condition in which the fundamental frequency is better transmitted to the periphery and thus louder, but the formative or formatives are not well transmitted, and therefore the vowel is not distinct, only louder than when heard over normal lung area.

RELATION OF HEMOGLOBIN, CELL COUNT AND CELL VOLUME TO THE ERYTHROCYTE SEDIMENTATION REACTION *

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In explaining the erythrocyte sedimentation reaction the majority of investigators have limited themselves to the study of the physicochemical changes that occur in the plasma and have paid little attention to the erythrocytes. The few attempts made to study the latter have been on citrated or oxalated blood. These anticoagulants, however, as will be shown later, so alter the quantitative relationship between the plasma and the cells as to render their use unsuitable for the study of the erythrocytes. The use of hirudin offers the best available means of keeping the blood in fluid condition without otherwise changing its composition. Its recent use by Gram and Norgaard [1] for the determination of standards of hemoglobin, cell count and cell volume for healthy persons suggested its employment in studying the same factors in conjunction with the sedimentation reaction.

METHODS

Specimens of blood were obtained before breakfast from ten healthy adults (physicians and nurses) and thirty patients in the following manner:

1. *Sedimentation Reaction.*—Into a sterile 2 cc. record syringe, a solution of 3.8 per cent sodium citrate was drawn to the 0.4 mark. With a tourniquet applied loosely above the elbow so as not to occlude the radial pulse, blood was aspirated from an arm vein to the 2 cc. mark, giving a dilution of 1:4. The syringe was then removed and, with the needle left in situ, about 5 cc. of blood was collected in a small bottle containing 1 mg. of powdered hirudin. The blood was thoroughly mixed. Citrated blood and duplicate samples of hirudinized blood were then drawn into 1 cc. serologic pipets approximately 20 cm. in length and graduated into hundredths. These were placed vertically in a specially constructed stand and kept firmly pressed by means of rubber bands on a rubber mat covering the base of the stand. The extent of

* From the Montefiore Hospital for Chronic Diseases.

1. Gram, H. C., and Norgaard, A.: Relation Between Hemoglobin, Cell Count and Cell Volume in Venous Blood of Normal Human Subjects, Arch. Int. Med. **31**:164-170 (Feb.) 1923.

sedimentation was noted at the end of one, two and twenty-four hours and the results read directly in percentage as recommended by Morriss.[2]

2. *Hemoglobin.*—On each specimen of hirudinized blood three determinations were made with a Sahli hemoglobinometer containing colored glass comparison rods. The hemoglobin percentage was calculated on the assumption that 100 per cent hemoglobin equals 5 millions of normal red blood cells so as to give a color index of 1. Gram and Norgaard [1] have shown that such a calculation differed on an average of 0.56 per cent from that obtained by correcting the hemoglobinometer to read on the basis of 100 per cent hemoglobin having an oxygen combining capacity of 18.5 per cent by volume. As a further check, however, the Sahli standard was compared with a standardized hemoglobin solution. The two methods checked closely.

3. *Cell Count.*—Two counts from separate pipets were made on each specimen of hirudinized blood. A Neubauer counting chamber and Haymen's diluting fluid were used for counting the cells. When differences between counts were greater than 200,000 cells, a third count was done.

4. *Cell Volume.*—Cell volume percentage was obtained with the use of Van Allen's [3] hematocrits. Four determinations on the blood of the controls and duplicate determinations on the blood of the patients were made. The tubes were centrifugalized for one to one and one-half hours at a rate of 2,400 revolutions per minute, the longer time being taken for the blood of the controls. The final readings did not include the layer of white blood cells. Variations that occurred between readings rarely exceeded 0.5 per cent.

RESULTS

The results will be discussed in the order in which they are tabulated.

Table 1 represents the data for ten normal subjects, five men and five women, with the comparative results of Gram and Norgaard. We obtained an average erythrocyte count of approximately 5 million cells as contrasted to the higher count of these authors and, as one would expect, our hemoglobin percentage is nearer 100. The volume index agrees closely with theirs, while the cell volume averages differ only by 0.66 per cent. Somewhat greater discrepancies are seen in comparing the data on the women, our averages being generally lower with the exception of that obtained on cell volume. It is quite possible that a larger group of controls would have compared more closely. The data listed in the remaining columns will be referred to in the general discussion of the results.

2. Morriss, W. H.: The Value of Erythrocyte Sedimentation Determinations in Pulmonary Tuberculosis, Am. Rev. Tuberc. **10**:431-440 (Dec.) 1924.

3. Van Allen, C. M.: An Hematocrit Method, J. Lab. & Clin. Med. **10**:1027-1040 (Sept.) 1925.

1. *Hemoglobin.*—The hemoglobin content of the red blood cells parallels to a certain degree the erythrocyte sedimentation reaction. However, there are a number of instances in which high hemoglobin percentages accompanied increased sedimentation as well as cases in which relatively low hemoglobin percentages accompanied normal sedimentation. In general, our results agree with those of Groedel and Hubert [4] who found that the lower the hemoglobin content of the blood the more frequently is increased sedimentation obtained and that the hemoglobin per se is not directly related either to the two or the twenty-four hour sedimentation values.

TABLE 1.—*Results of Blood Tests of Ten Normal Subjects*

	Number	Age	Hemoglobin, per Cent	Cell Count, Millions per C.Mm.	Volume Index	Cell Volume, per Cent (Hematocrit)	Sedimentation Reaction on Hirudinized Blood, per Cent: 2 Hrs.	Sedimentation Reaction on Hirudinized Blood, per Cent: 24 Hrs.	Cell Volume, per Cent (Spontaneous)	Cell Volume (Spontaneous) Minus Cell Volume (Hematocrit)	Cell Volume (Hematocrit) / Cell Volume (Spontaneous)	2-Hour Reading / 24-Hour Reading × 100
			(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
Normal males	1	25	106	5.600	0.97	51.0	2.0	29.5	70.5	19.5	0.72	6.8
	2	25	110	5.250	1.03	50.5	3.5	32.5	67.5	17.0	0.75	10.8
	3	25	100	4.750	1.05	47.0	6.0	35.0	65.0	18.0	0.72	17.1
	4	25	84	4.550	0.94	40.0	10.5	39.5	60.5	20.5	0.66	26.6
	5	25	100	4.900	1.00	46.5	13.0	41.0	59.0	12.5	0.79	31.7
Average			100	5.010	1.00	47.0	7.0	35.5	64.5	17.5	0.73	18.6
Maximum			110	5.600	1.05	51.0	13.0	41.0	70.5	20.5	0.79	31.7
Minimum			84	4.550	0.94	40.0	2.0	29.5	59.0	12.5	0.66	6.8
Gram and Norgaard (10 Cases)												
Average			108.32	5.454	1.00	46.34						
Maximum			117.26	5.911	1.03	50.00						
Minimum			95.62	4.854	0.96	42.38						
Normal females	6	35	81	3.750	1.14	40.0	10.0	41.5	58.5	18.5	0.68	24.1
	7	21	84	3.650	1.24	42.5	12.0	44.5	55.5	13.0	0.77	26.9
	8	23	82	4.200	1.08	42.5	21.0	46.0	54.0	11.5	0.79	45.7
	9	23	94	4.100	1.10	42.5	20.0	46.0	54.0	11.5	0.79	43.5
	10	22	87	4.100	1.12	43.0	22.5	46.0	54.0	11.0	0.80	48.9
Average			86	3.960	1.13	42.1	17.1	44.8	55.2	13.1	0.77	37.5
Maximum			94	4.200	1.24	43.0	22.5	46.0	58.5	18.5	0.80	48.9
Minimum			82	3.650	1.08	40.0	10.0	41.5	54.0	11.0	0.68	24.1
Gram and Norgaard (10 Cases)												
Average			93.85	4.654	1.01	40.53						
Maximum			101.68	5.054	1.06	43.00						
Minimum			87.49	4.360	0.97	38.81						

2. *Cell Count.*—What has been stated regarding the relationship of hemoglobin to the sedimentation reaction applies equally to the cell count. The patients in this series had chronic diseases with varying degrees of secondary anemia so that with the decrease in cell count there was a proportional decrease in the hemoglobin content.

4. Groedel, F. M., and Hubert, G.: Die Blutkörperchensenkungsgeschwindigkeit bei inneren Erkrankungen, besonders bei Kreislaufstörungen, Ztschr. f. klin. Med. **192**:31-46 (April) 1925.

TABLE 2.—*Results of Blood Tests of Patients*

Number	Age	Sex*	Clinical Diagnosis	Hemoglobin, per Cent	Cell Count, Millions per C.Mm.	Volume Index	Cell Volume, per Cent (Hematocrit)	Sedimentation Reaction on Hirudinized Blood, per Cent: 2 Hrs.	Sedimentation Reaction on Hirudinized Blood, per Cent: 24 Hrs.	Cell Volume, per Cent (Spontaneous)	Cell Volume (Spontaneous) Minus Cell Volume (Hematocrit)	Cell Volume (Hematocrit) / Cell Volume (Spontaneous)	2-Hour Reading / 24-Hour Reading × 100
				(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
11	47	♂	Multiple sclerosis	107	5.200	1.02	50.0	17.5	38.5	61.5	11.5	0.81	45.4
12	58	♂	Resection of cancer of rectum; no evidence of recurrence	98	4.150	1.24	48.5	12.5	39.0	61.0	12.5	0.79	32.0
13	17	♂	Chronic rheumatic heart disease	102	4.750	1.06	47.5	11.5	41.5	58.5	11.0	0.81	27.7
14	57	♂	Chronic arthritis	90	4.500	1.12	47.5	13.0	42.0	58.0	10.5	0.82	30.9
15	52	♂	Cancer of stomach	105	4.900	1.08	50.0	28.0	43.5	56.5	6.5	0.88	64.4
16	60	♂	Generalized arteriosclerosis	101	5.000	1.01	47.5	27.5	43.5	56.5	9.0	0.84	63.2
17	76	♂	Generalized arteriosclerosis	89	3.950	1.32	49.0	24.0	44.0	56.0	7.0	0.88	54.5
18	39	♀	Paralysis agitans (postencephalitic)	80	4.100	1.11	43.0	13.5	44.0	56.0	13.0	0.77	30.7
19	49	♂	Chronic pulmonary tuberculosis	88	3.800	1.23	44.0	37.0	46.0	54.0	10.0	0.81	80.4
20	20	♀	Chronic pulmonary tuberculosis	88	4.200	1.07	42.5	39.0	49.0	51.0	8.5	0.83	79.6
21	46	♂	Endothelioma of right pleura with skeletal metastases	87	4.750	0.96	43.0	41.5	49.5	50.5	7.5	0.85	83.8
22	64	♀	Cancer of bladder	88	4.100	1.06	41.0	35.0	51.0	49.0	8.0	0.84	68.6
23	39	♀	Paralysis agitans (postencephalitic)	81	3.750	1.15	40.5	45.5	52.0	48.0	7.5	0.84	87.5
24	64	♂	Chronic osteomyelitis of left femur	77	4.150	1.05	41.0	42.0	52.5	47.5	6.5	0.86	80.0
25	56	♂	Tabes dorsalis	88	4.150	0.97	38.0	43.5	53.0	47.0	9.0	0.81	82.1
26	37	♂	Paralysis agitans (postencephalitic)	79	3.600	1.15	39.0	33.0	53.0	47.0	8.0	0.83	62.3
27	69	♀	Cancer of external genitalia	86	3.670	1.21	41.5	48.0	53.0	47.0	5.5	0.88	90.6
28	30	♂	Chronic pneumonitis with adherent pleura	70	4.300	0.91	37.0	43.5	54.5	45.5	8.5	0.81	79.8
29	52	♂	Chronic pulmonary tuberculosis	74	3.550	1.09	36.5	46.0	55.5	44.5	8.0	0.82	82.9
30	29	♀	Myelitis due to Pott's disease	80	3.600	1.11	37.5	50.0	56.0	44.0	6.5	0.85	89.3
31	32	♀	Infectious polyarthritis	76	3.750	1.08	38.0	52.0	56.5	43.5	5.5	0.87	92.0
32	61	♀	Undiagnosed	81	3.350	1.17	37.0	44.5	58.0	42.0	5.0	0.88	76.7
33	60	♀	Cancer of breast with metastases to lung	77	3.800	1.05	37.5	53.5	58.0	42.0	4.5	0.89	92.2
34	65	♀	Basal cell epithelioma of lip with bone necrosis	84	4.000	0.96	34.0	53.0	58.0	42.0	8.0	0.81	91.4
35	37	♀	Cancer of breast with skeletal metastases	81	4.000	0.93	35.0	56.0	59.5	40.5	5.5	0.86	94.1
36	63	♂	Cancer of esophagus	80	3.550	1.05	35.0	55.0	60.0	40.0	5.0	0.88	91.7
37	21	♀	Advanced pulmonary tuberculosis	66	3.800	0.92	33.0	58.0	62.0	38.0	5.0	0.87	93.5
38	40	♂	Cancer of colon	52	2.150	1.33	27.0	63.0	68.0	32.0	5.0	0.84	92.6
39	62	♀	Cancer of colon	45	2.100	1.06	21.0	72.0	75.0	25.0	4.0	0.84	96.0
40	70	♂	Cancer of bladder	44	2.150	0.94	19.0	74.0	76.5	23.5	4.5	0.81	96.7

* In this and table 3, ♂ indicates male, ♀ female.

3. *Volume Index.*—The volume index was calculated by dividing the cell volume by the red count in millions per cubic millimeter and multiplying this by the factor 0.1066 in order to obtain 1.0 as the normal volume index. This index shows no significant relationship to the erythrocyte sedimentation reaction. The color index also was calculated and was found to parallel the volume index to some extent.

4. *Cell Volume.*—The cell volume is an expression of the cell count and the hemoglobin content of the cells and is an excellent check on them. When the cell volume readings are compared with the sedimentation reaction, the relationship between the two, especially the twenty-four hour readings of the latter, is quite striking. It will be noted that with a decrease in cell volume there is a proportional increase in the twenty-four hour reading. Since the sedimentation results are expressed in plasma percentage rather than in terms of cells, column 7 is inserted to show this relationship on a comparative basis. In other words, the cell volume obtained by hematocrit bears a fixed ratio to that obtained by the spontaneous settling of the cells in twenty-four hours, which represents approximately the limit to which the cells can settle. In the subsequent two columns this fact is illustrated more clearly. Thus, in spite of the greater differences between the spontaneous and centrifugal settling of the cells that occurs in the blood showing normal or moderate sedimentation, and represented in column 8, the ratio $\frac{\text{cell volume per cent (hematocrit)}}{\text{cell volume per cent (spontaneous)}}$ averages 83.9 for the patients and 74 for the controls. The lower factor of the healthy controls is in itself of some interest. From it we deduce that the red blood cells of healthy persons possess a greater faculty of remaining in suspension than the blood of patients. This may be another expression of the relative stability of the blood in health. That this occurs in spite of cell volume percentages that are frequently lower than that in patients' blood adds greater significance to this finding.

The two hour readings do not bear as close a relationship to the cell volume as do the twenty-four hour readings, although there is a distinct tendency for them to do so. This we believe is due to the fact that in the early hours of sedimentation two forces are at work, the plasma and the cells. In a previous article one of us [5] has shown that both plasma and serum show increasing ability of precipitation on the addition of various reagents, and that this characteristic of the plasma and serum paralleled the erythrocyte sedimentation reaction. It may be assumed, therefore, that in the early hours of sedimentation there is greater freedom of interplay between plasma and cells but that as

5. Rubin, E. H.: Relation of the Erythrocyte Sedimentation Reaction to the Ability of Flocculation of the Plasma and Serum, Arch. Int. Med. **37**:848 (June) 1926.

sedimentation progresses the mechanical force exerted by the cells becomes increasingly greater until, in time, the reaction becomes predominantly an expression of cell volume. We have devised the ratio $\frac{\text{two hour sedimentation per cent}}{\text{twenty-four hour sedimentation per cent}}$ to express the relationship existing between plasma and cells at periods when they exert their greatest influence on sedimentation. This index has the advantage over the two hour reading alone, which is generally employed, because it takes into consideration such cases in which a normal corpuscular volume exists in the presence of a pathologic condition of the tissue as well as cases in which the degree of anemia is out of proportion to the severity of tissue destruction. Both types of cases are frequent.

Applying the index thus obtained to the forty cases studied we find all the normals and five patients giving values below 50, that is, the sedimentation at two hours is less than one-half that occurring in twenty-four hours while the remaining twenty-five patients have values over 50, of which ten are over 90.

EFFECT OF ANTICOAGULANTS ON CELL VOLUME

The difficulty of demonstrating a relationship between the sedimentation reaction and the cell volume with the use of citrates or oxalates is easily explained and has been recognized by Westergren [6] and several others. Thus, for example, when the usual dilution of 1 part sodium citrate is added to 4 parts of whole blood containing 50 per cent cells, the cells become only 40 per cent of the citrated blood, with a loss of 10 per cent; if, however, the cells make up only 20 per cent of the blood volume, the cells become reduced to 16 per cent of the citrated blood, with a loss of 4 per cent. The determination of cell volume on citrated blood is therefore erroneous unless the factor of dilution is taken into consideration, assuming that the diluent is isotonic with blood. A preliminary study of forty-six cases convinced us of the inaccuracy inherent in such a method. The tendency, however, for increased sedimentation to accompany low cell volume was so evident grossly that it seemed desirable to study the question further.

With the use of salts to prevent coagulation, changes are produced in the osmotic pressure of the blood which cause considerable alteration in the size of the cells. Hooper, Smith, Belt and Whipple [7] have shown that the addition of sodium oxalate crystals in minimal amounts capable of preventing coagulation (0.1 per cent) will cause on an average a shrinkage in the cell volume of about 3 per cent, while Van Allen [3] found the change to be 5.5 per cent. We have found the shrinkage to vary

6. Westergren, A.: Die Senkungsreaktion, Ergebn. d. inn. Med. u. Kinderh. **26**:577-732 (April) 1924.

7. Hooper, C. W., Smith, H. P., Belt, A. E., and Whipple, G. H.: Blood Volume Studies, Am. J. Physiol. **51**:205-220 (March) 1920.

somewhat with the cell volume, the greater the cell volume the greater being the shrinkage, as compared to the cell volume obtained on hirudinized blood. Since it is customary to add an excess of the oxalate in actual practice, this source of error becomes much larger. Table 3 represents four simultaneous cell volume determinations on hirudinized, citrated and oxalated blood to illustrate the matter under discussion.

Using graded amounts of citrate solution to compensate for variations in cell volume, Bönniger and Herrmann [8] were able to show a relationship between the sedimentation reaction and the cell volume. The use of hirudin in our experiments simplified matters by dispensing with extraneous diluents. The action of hirudin in preventing coagulation is yet to be determined but it is certain that for our purpose it offered an excellent means of studying the problem

EFFECT OF DILUTION AND CONCENTRATION ON THE SEDIMENTATION REACTION

The relationship that exists between cell volume and the sedimentation reaction can be demonstrated also by changing the proportion of plasma to cells. This is easily done by adding or subtracting definite

TABLE 3.—*Effect of Anticoagulants on Cell Volume*

Number	Age	Sex	Clinical Diagnosis	1 Mg. Hirudin in 5 Cc. Blood, Hematocrit Cell Volume, per Cent	1:4 Dilution with 3.8 per Cent Sodium Citrate Solution, Hematocrit Cell Volume, per Cent	5 Mg. Sodium Oxalate in 5 Cc. Blood, Hematocrit Cell Volume, per Cent
1	47	♂	Multiple sclerosis	51	38	48
2	57	♂	Chronic arthritis	44.5	31.5	41.5
3	40	♀	Pulmonary tuberculosis	35	24.5	33
4	70	♂	Cancer of bladder	20	15.5	20

amounts of homologous plasma and determining the cell volume on the modified samples by hematocrit. The method besides being simple has the further advantage of keeping the original cell volume intact. In view of the work of Abderhalden [9] who found that the various layers of erythrocytes differ in their sedimenting properties, this precaution was worth taking.

PROTOCOLS

EXPERIMENT 1.—The hirudinized blood of a control was divided evenly into four small test tubes. On one of these, A1, the routine sedimentation, cell volume, hemoglobin and cell count determinations were made. The blood in

8. Bönniger, M., and Herrmann, W.: Blutkörperchen-Senkungsgeschwindigkeit und Volumen, Klin. Wchnschr. **2**:744-745 (April) 1923.

9. Abderhalden, E.: Weitere Forschungen über die Senkungsgeschwindigkeit der roten Blutkörperchen bei gleichen und bei verschiedenen Tierarten und unter verschiedenen Bedingungen (ii. Mitteilung), Pflüger's Arch. f. d. ges. Physiol. **193**:236-280, 1922.

the remaining three tubes was allowed to sediment spontaneously and when the cells had settled sufficiently to show clear plasma, 0.35 cc. of plasma was removed from one tube, A4, and immediately added to a second tube, A3. On these modified samples, as well as on the fourth sample that served as a control, the same determinations were made as on the first. Frequent readings were taken to plot the results.

It was noted that when plasma was added to tube A3 thereby producing a relative decrease in cell volume there followed an increased sedimentation; that tube A4 in which the cell volume was relatively increased gave a reaction above normal, and that tube A2, the control, gave approximately the same sedimentation as the original, A1.

Experiment 2.—The same procedure used in experiment 1 was carried out on the blood of a tuberculous patient showing an increased sedimentation reaction. B1 is the sedimentation graph of the original blood, B2 and B3, from which 0.5 and 0.75 cc. samples of plasma were removed, respectively, while B4 represents the graph of a sample of blood diluted with 1 cc. of plasma.

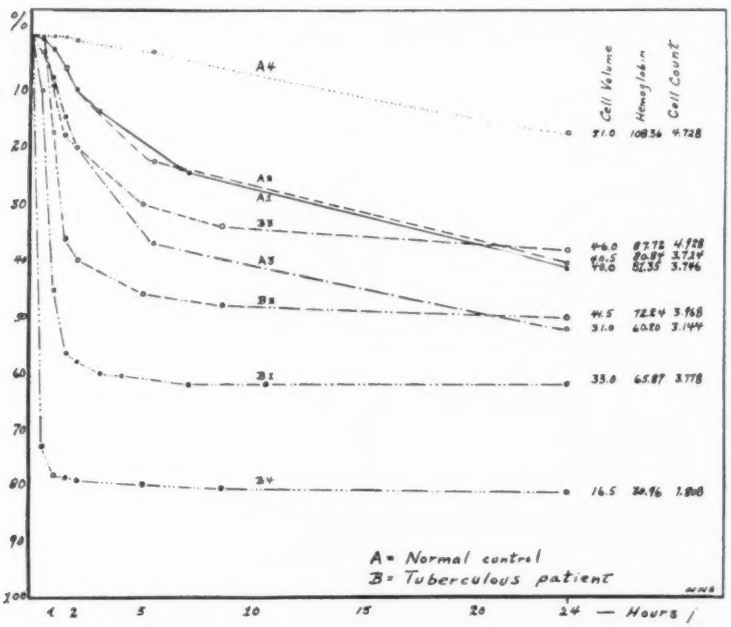

Chart 1.—Effect of dilution and concentration on the sedimentation reaction.

Depending on the degree of dilution or concentration of the erythrocytes, a relative increase or decrease in the sedimentation reaction was obtained. The changes affected the two hour as well as the twenty-four hour readings. The hemoglobin percentages and cell counts roughly followed the alterations in cell volume.

These experiments were repeated. The results substantiate our previous opinion regarding the relationship of plasma to cells and the desirability of combining both factors in some such manner as we have indicated.

Clinically, it is well known that in conditions associated with polycythemia a delay is found while marked anemia causes an increase in the sedimentation reaction. In view of our results, we believe that the volume of erythrocytes exerts an important influence on the sedimentation reaction under all conditions, and that possibly physiologic changes in sedimentation are also to a great extent influenced by the erythrocyte factor. Thus, the lower cell volume percentage found in women would

TABLE 4.—*Comparison of Sedimentation Between Citrated and Hirudinized Blood*

	Two Hour Sedimentation, per Cent			Twenty-Four Hour Sedimentation, per Cent		
Case	Citrated Blood	Hirudinized Blood	Difference	Citrated Blood	Hirudinized Blood	Difference
1	1.0	2.0	— 1.00	18.5	29.5	—11.0
2	1.75	3.5	— 1.75	22.5	32.5	—10.0
3	2.5	6.0	— 3.5	27.0	35.0	— 8.0
6	6.0	10.0	— 4.0	40.5	41.5	— 1.0
13	6.0	11.5	— 5.5	35.5	41.5	— 6.0
4	7.0	10.5	— 3.5	40.5	39.5	+ 1.0
12	7.0	12.5	— 5.5	32.0	39.0	— 7.0
9	7.5	20.0	—12.5	40.0	46.0	— 6.0
14	7.5	13.0	— 5.5	34.5	42.0	— 7.5
5	8.0	13.0	— 5.0	35.0	41.0	— 6.0
11	8.0	17.5	— 9.5	34.5	38.5	— 4.0
18	8.0	13.5	— 5.5	39.0	44.0	— 5.0
7	10.0	12.0	— 2.0	41.0	44.5	— 3.5
17	10.0	24.0	—14.0	38.0	44.0	— 6.0
8	13.0	21.0	— 8.0	42.0	46.0	— 4.0
10	15.0	22.5	— 7.5	46.0	46.0	± 0.0
15	15.0	28.0	—13.0	44.0	43.5	+ 0.5
16	16.0	27.5	—11.5	42.5	43.5	— 1.0
20	20.0	33.0	—13.0	50.0	53.0	— 3.0
22	26.0	35.0	— 9.0	56.5	51.0	+ 5.5
32	26.5	44.5	—18.0	56.5	58.0	— 1.5
23	29.0	43.5	—14.5	55.0	53.0	+ 2.0
30	35.0	50.0	—15.0	61.0	56.0	+ 5.0
26	38.0	39.0	— 1.0	60.0	49.0	+11.0
27	40.0	48.0	— 8.0	60.0	53.0	+ 7.0
28	41.0	53.5	—12.5	65.0	58.0	+ 7.0
34	48.0	53.0	— 5.0	65.0	58.0	+ 7.0
24	49.0	45.5	+ 3.5	65.0	52.0	+13.0
31	50.0	52.0	— 2.0	65.5	56.5	+ 9.0
19	53.0	37.0	+16.0	63.0	46.0	+17.0
25	53.0	43.5	+ 9.5	60.0	54.5	+11.5
21	53.5	42.0	+11.5	67.0	52.5	+14.5
38	54.0	63.0	— 9.0	74.0	68.0	+14.0
35	55.0	56.0	— 1.0	68.5	59.5	+ 9.0
36	55.0	55.0	± 0.0	69.5	60.0	+ 9.5
21	56.0	41.5	+14.5	64.0	49.5	+14.5
29	60.0	46.0	+14.0	68.0	55.5	+12.5
39	65.0	72.0	— 7.0	78.5	75.0	+ 3.5
37	68.5	58.0	+10.5	72.0	62.0	+10.0
40	82.0	74.0	+ 8.0	84.0	76.5	+ 7.5

explain the greater sedimentation reactions that they give in comparison to men. The clinical value of the sedimentation reaction in surgery has been reviewed by one of us [10] in another article and a micromethod [11] described to facilitate a wider application of the test.

10. Rubin, E. H.: The Clinical Value of the Erythrocyte Sedimentation Reaction in Surgery, Surg. Gynec. Obst. **42**:652-656 (May) 1926.

11. Morriss, W. H., and Rubin, E. H.: The Sedimentation Reaction of Erythrocytes: Clinical Applications and a Micromethod, J. Lab. & Clin. Med. **11**:1045-1052 (Aug.) 1926.

COMPARISON OF SEDIMENTATION BETWEEN CITRATED AND HIRUDINIZED BLOOD

In table 4 are arranged the two and twenty-four hour readings on the citrated and hirudinized blood. The one hour reading is omitted because of the inaccuracy in taking readings on a rapidly sedimenting blood at this time.

Table 4 shows that approximately up to the 15 per cent reading on the citrated column the hirudinized blood settles more rapidly and maintains its lead to the end of the twenty-four hours. From the 15 to the 29 per cent reading, the twenty-four hour results show occasionally a greater sedimentation of the citrated blood and that above the 29 per cent reading the citrated blood had definitely outpaced the hirudinized blood

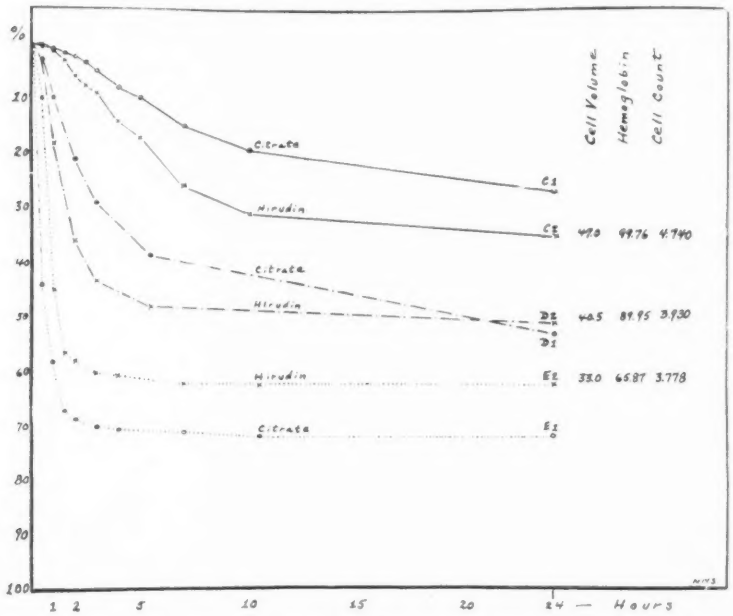

Chart 2.—Relation between sedimentation reaction on citrated and hirudinized blood.

at some period between the second and the twenty-fourth hour. Above the 50 per cent reading we note that in many instances as early as the second hour, the citrated blood had settled faster and had retained its lead over the hirudinized blood throughout the twenty-four hours.

This relationship is made clearer in chart 2.

To interpret this apparently paradoxic relationship of the citrated to the hirudinized blood, we have the following explanation to offer, which, however, is an hypothesis:

When sodium citrate is added to make a dilution of 1 : 4 of blood that contains, let us assume, 50 per cent plasma and 50 per cent cells, the plasma and the cells are diluted the same amount, each forming two-fifths of the entire mixture. Such a relationship might exist when citrate is added to blood with a normal cell volume. The diluent, if isotonic, produces relatively little change on the cells in comparison to the effect it exerts on the plasma. If the plasma has low precipitating ability, as is usually the case in normal blood, the additional citrate is sufficient to reduce this power to some degree with consequent neutralization of whatever action it may exert on the cells. The hirudinized blood, on the contrary, unaffected by diluent acts in maximum intensity and causes a greater sedimentation of the cells.

If, however, the blood contains 75 per cent plasma and 25 per cent cells, on the addition of citrate in the dilution of 1 : 4 the plasma is converted to three-fifths of the entire mixture and is therefore in a relatively more concentrated form than in the case previously described. Such a plasma would have greater precipitating power on a mathematical basis. In reality, we find the plasma in these cases to possess much greater ability of precipitation than normally. This would manifest itself in increased sedimentation.

The cell volume is affected by the addition of diluent in the following manner: In the first case, the modified plasma acts on cells totaling two-fifths of the entire mixture; in the second case, on cells totaling only one-fifth of the entire mixture. Thus, we have a relatively less concentrated plasma acting on a greater cell volume in the first case and just the reverse in the second case although the citrate is added to both in the same proportion. This fact, in our opinion, is sufficient to account for the greater sedimentation occurring in the latter instance. A consideration of these two factors might help explain the differences observed between the sedimentation of the citrated and the hirudinized blood and to a certain degree the mechanism of sedimentation in the former. We are assuming that the hirudin, itself, is not playing a significant part in the reaction aside from its action as an anticoagulant.

SUMMARY

1. The erythrocyte sedimentation reaction was studied on hirudinized blood.

2. This method obviates such complicating factors as dilution and osmotic pressure changes that are inherent in the methods employing citrates or oxalates to prevent coagulation.

3. With the use of hirudinized blood, a close relationship was found to exist between the erythrocyte sedimentation reaction and the cell volume as determined by hematocrit, the lower the cell volume, the greater being the rapidity of sedimentation.

4. By artificially decreasing the cell volume normally sedimenting blood could be made to settle more rapidly. Conversely, by increasing the cell volume, rapidly sedimenting blood could be made to approach normal limits.

5. Comparative results with hirudinized blood showed that the use of 3.8 per cent sodium citrate solution as an anticoagulant in the dilution of 1:4 of blood gave inconstant readings in the sedimentation reaction. These changes seemed referable to the effect of the diluent on the plasma and the cells.

6. The stability of hirudinized blood seems better expressed by the ratio $\frac{\text{two hour}}{\text{twenty-four hour}}$ reading than by the two hour reading alone, as this ratio expresses the relationship of plasma to cells at periods when they exert their greatest influence on sedimentation.

Book Reviews

AVIATION MEDICINE. LOUIS HOPEWELL BAUER, M.D., Major, M.C., U. S. A., Commandant of the School of Aviation Medicine. Price, $7.50. Pp. 241. Baltimore: Williams & Wilkins Co., 1926.

With the great development of aviation during the late war, it became necessary for the advancement of aviation as a military measure and for the safety of those engaged in it, to study the physical requirements for successful flying and for the performance of the various military duties required. This demanded a thorough study of the physiologic response to altitude. To accomplish this the government maintained a research laboratory in which a large amount of work was done on the physiologic response to altitude, visual requirements for flying, the mechanism of equilibration, the psychologic reactions under different conditions, reaction time and the various bodily requirements. In addition, the experiences in the flying fields in this country and abroad and in the combat zone were collected and analyzed.

In the course of such investigations, it was inevitable that important contributions should be made to our fundamental knowledge. All of this material has been available to the author, who has been commandant at the School of Aviation Medicine since shortly after the armistice, and to whose wise direction much of the credit for the work accomplished should be given.

The book is primarily a text for aviation surgeons and for those interested in civilian aviation, and as such is of exceptional value. It is much more than that, however, and is valuable to those interested in every branch of medicine. While the results of investigation and experience here and abroad must be considered briefly at times, full references are given to the original literature.

The general physical needs are considered, followed by chapters on the visual requirements, the nose, throat and ear, psychologic requirements, reaction time and methods for testing the applicant. A full section is devoted to the physiology of aviation, especially the effect of altitude on the circulation and respiration and the methods by which the body compensates, the psychologic effects of altitude, and methods for determining the reaction of the applicant to altitude. In a section of several chapters the care of the aviator is discussed, with chapters on fatigue, staleness, protective devices and a chapter devoted to airplane accidents. A final chapter is devoted to civilian aviation. Details of tests and examination with specimen charts are given in a supplement.

A complete bibliography adds to its great value as a text and book of reference.

DIE ELECTROGRAPHIE, UND ANDERE GRAPHISHE METHODEN IN DER KREISLAUFDIAGNOSTIK. DR. ARTHUR WEBER, A. O. Professor in der Universität Giessen. Price, 19 marks. Pp. 208, with 139 illustrations. Berlin: Julius Springer, 1926.

This volume is a compact and a thorough exposition of methods for the graphic registration of the phenomena associated with the heart beat, and as a text and book of reference, it fills a place not filled by any one book in English. The apparatus necessary for the establishing of a laboratory for the investigation of heart disease is considered in general; the Frank capsule, photographic registration and application to the registration of the venous or arterial pulse, respiration, or the heart sounds. The electrocardiograph and its use are described in some detail.

The bulk of the book deals with the results which may be obtained by the use of the apparatus described, and with what may be learned from the results. The normal observations are described and the deviations from normal in different physiologic and anatomic conditions. The arrhythmias are given

under a classification based on the properties of heart muscle as described by Hering, such as disturbances of stimulus production, of stimulus conduction, of excitability and of contractility, and the observations by all the methods of investigation described are considered. The pathologic background, experimental production, physiologic theories and clinical significance are discussed. Auricular fibrillation is discussed under the heading "Disturbances of Stimulus Conduction," as arrhythmia absoluta. As is warranted by its relative importance, more space is given to the theories of its production than to the other arrhythmias. Pulsus alternans is presented under "Disturbances of Contractility." As in the case of the discussion on auricular fibrillation, the different theories of its production are given fairly and impartially, just how much so depending on the views of the reader.

The book is of value to students and clinicians. There is a full bibliography.

It may not be amiss to voice the regret that more students do not have the reading knowledge of foreign languages to make such volumes as useful to them as they should be.

GESUNDHEITSPFLEGE IM MITTELALTERLICHEN BASEL. By PROF. KARL BAAS, M.D. Pp. 120. Zurich: Fussle, 1926.

DIE STAATLICHE PESTPROPHYLAXE IM ALTEN ZURICH. DR. ARNOLD TREICHLER. Pp. 28. Zurich: Fussle, 1926.

Both treatises, which form part of a series of studies dealing with the local medical history of Zurich edited by Dr. Wehrli, offer a vivid picture of medieval medicine and the gradual development of state control of epidemics and public health in general. The treatise of Baas is particularly rich in material relating to the guild organization of the surgeons, the regulation of midwives and the development of the city hospitals. Both studies will form an interesting addition to the medico historical library.

LES MEDICAMENTS CARDIAQUES. By L. CHEINISSE. Price, 14 francs. Paris: Masson et Cie, 1925.

Dr. Cheinisse has collected a vast amount of information from the literature of all countries, has sifted and analyzed the facts given, and in this volume gives the present status of all of the drugs used in the treatment of cardiac disease.

He considers in turn the source of the medicine, its various preparations and modes of administration with a critical discussion of their indications and relative values, the indications and contraindications for use of the drug itself, its therapeutic, pharmacologic and physiologic effects and its effects when administered with other drugs.

He considers digitalis, strophanthus (strophanthine and ouabain) squills, sparteine, adonis vernalis, as well as several less known drugs, as specifics, and calcium chloride, caffeine, camphorated oil, epinephrine, atropine, eserine, quinine and quinidine as adjuncts, ending with chapters on cardiac opotherapy and intracardiac injections.

The book is simply and clearly written, and accomplishes well the aims of the writer.

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